What efficacy and safety profiles can we expect

Mathematical and Computational Biology in Drug Discovery (MCBDD) Module IV

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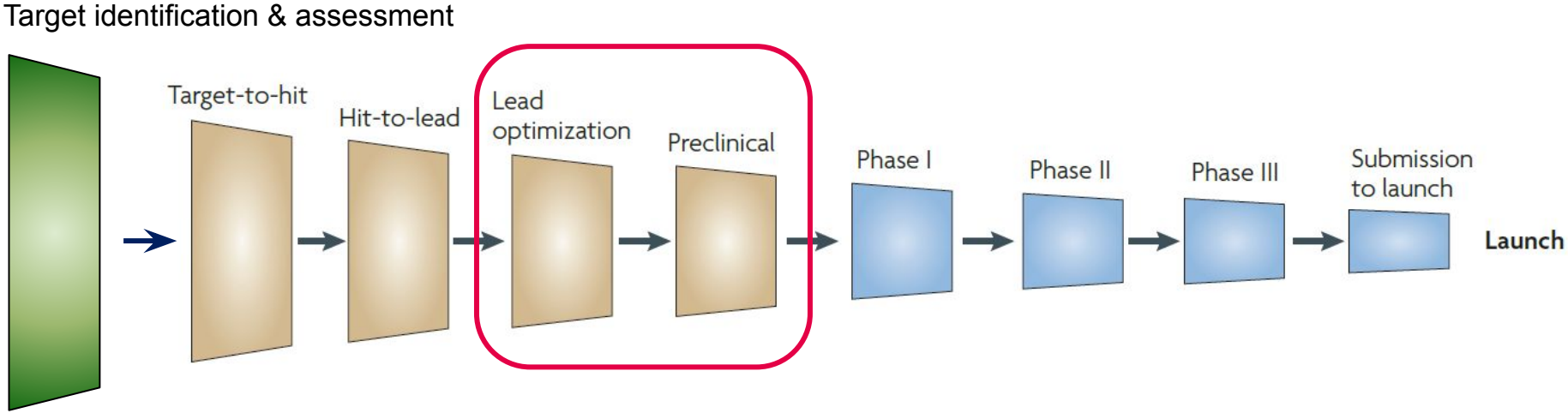
May 2025

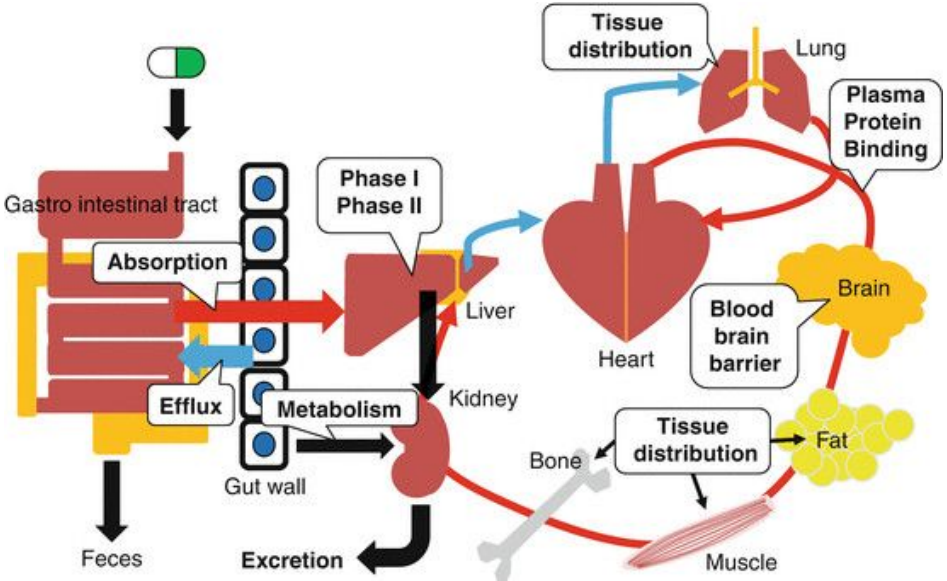
Where are we now



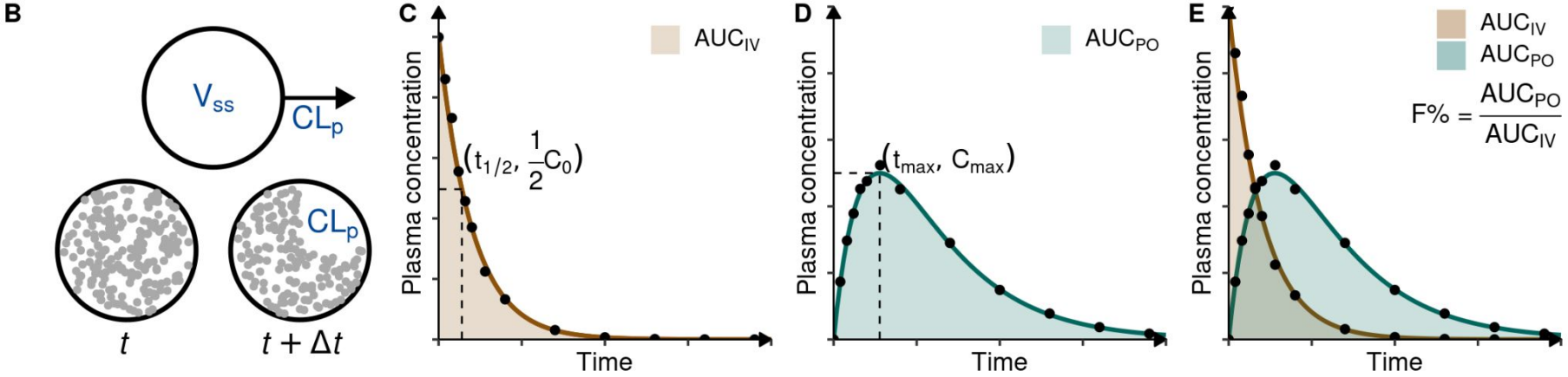
Goal: we want to select **one compound** from a few (~10^2-10^0) for entry in human.

Key factors to consider in selecting compounds

- **Efficacy and pharmacodynamics(PD)**
- **Pharmacokinetics (PK)**
  - Absorption
  - Distribution
  - Metabolism
  - Excretion
- **Toxicology**



Key PK parameters: Vss, CLp, $t_{1/2}$, t_{max}, C_{max}, and F



Vss	Volume of distribution at steady state.	**C_{max}**	Maximum plasma concentration.
CLp	Plasma clearance.	**t_{max}**	Time point in which the Cmax is measured.
$t_{1/2}$	Half-life, time for a substance to reach the half concentration of the initial value (C_0).	**F% (or F)**	Bioavailability, the percentage of the administered compound reaching systemic circulation.

The Hill function as a a typical PD model

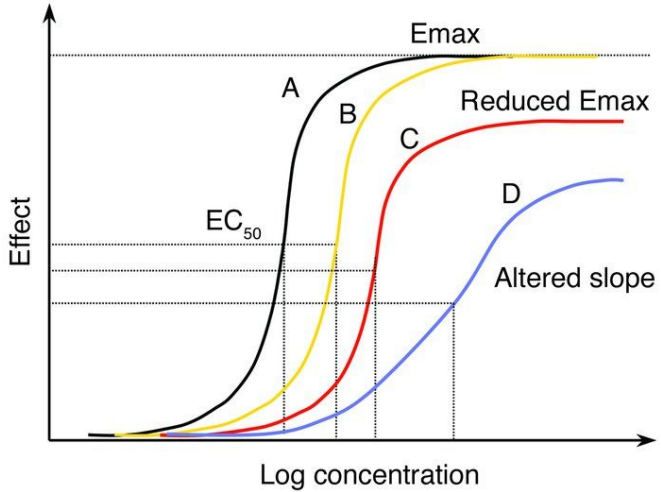
- The Hill function is one of the mostly useful non-linear functions to model biological systems.
- In its general form, H_{max} indicates the maximal value to which the function is asymptotic, *n* is the shape parameter (known as the Hill's coefficient), and *k* is the reflection point, often abbreviated as XC_{50} (X=I, E, C, …), the half-saturation constant.
- The Michaelis-Menten model is a special case of the Hill function with *n=1*.

$$H = H_{max}\frac{x^n}{k^n + x^n}$$

General form of the Hill function

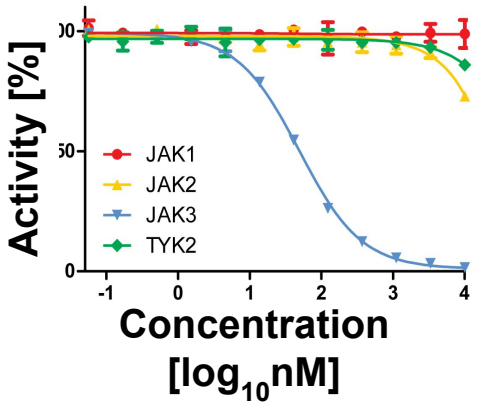
$$E = E_{max}\frac{[L]^n}{EC_{50}^n + [L]^n} = E_{max}\frac{1}{1 + (\frac{EC_{50}}{[L]})^n}$$

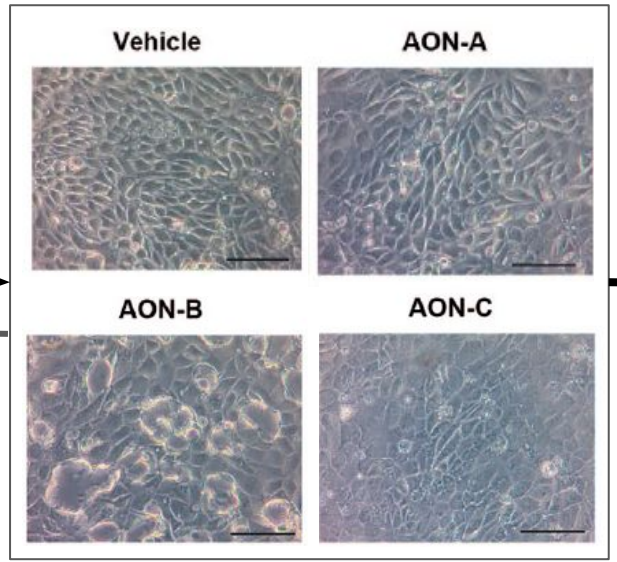
Modelling dose-dependent effect

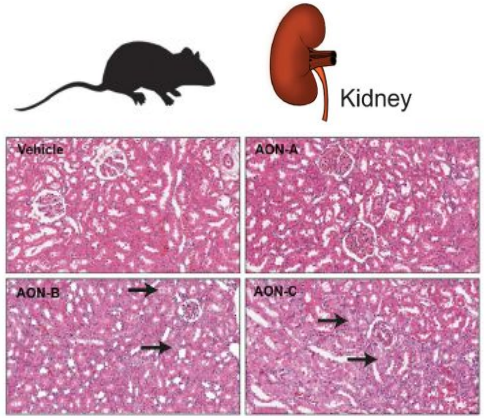


White. *J Clin Invest.* 2004;113(8):1084-1092. https://doi.org/10.1172/JCI21682.

Classical workflow of efficacy and toxicity assessment







Biochemical & biophysical assays

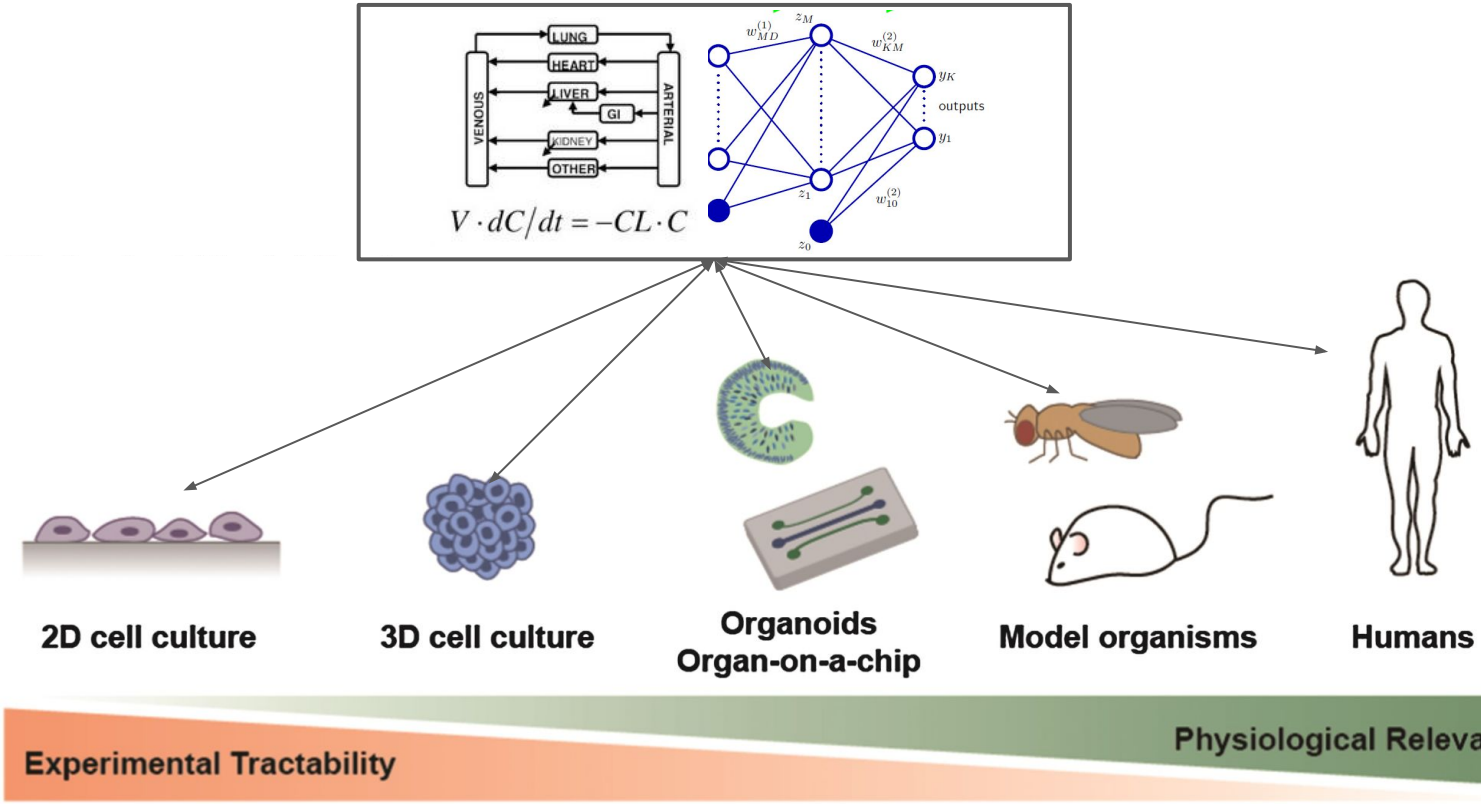
Cellular assays (*in vitro*)

Animal experiments (*in vivo*)

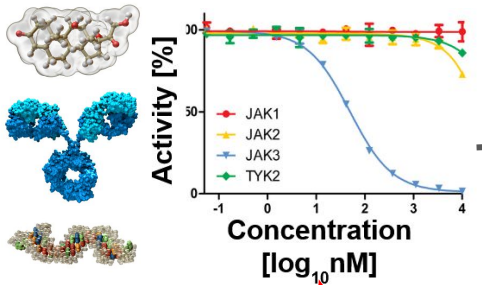
Usual workflow

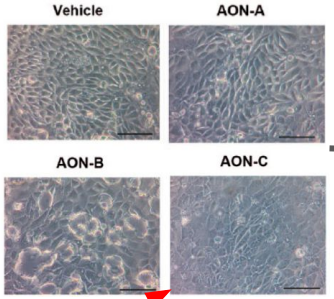
Assay development

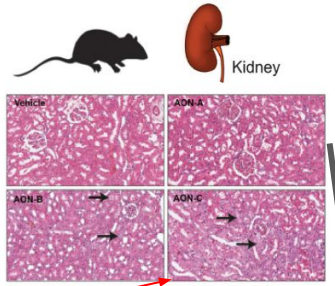
Biological and computational models of human diseases



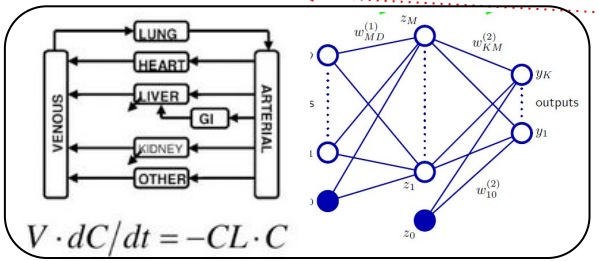
Computational methods empower efficacy and toxicity assessment





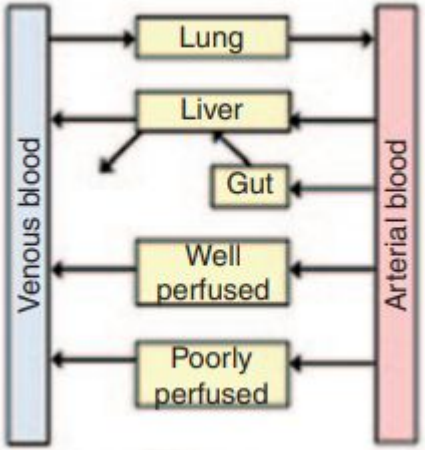


High-throughput technologies (omics, microscopy, etc.)

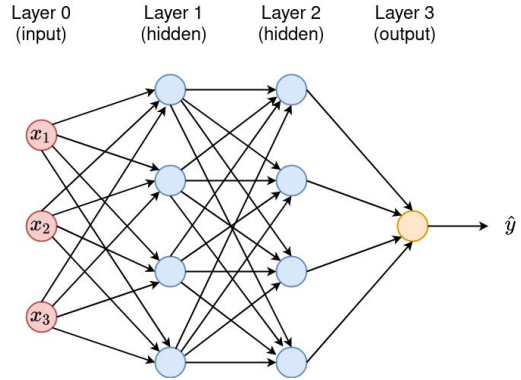


Mechanistic, causal, and statistical models

Three types of computational models

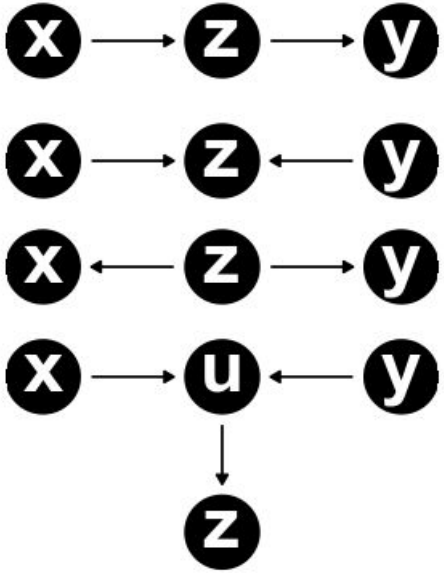


Mechanistic models



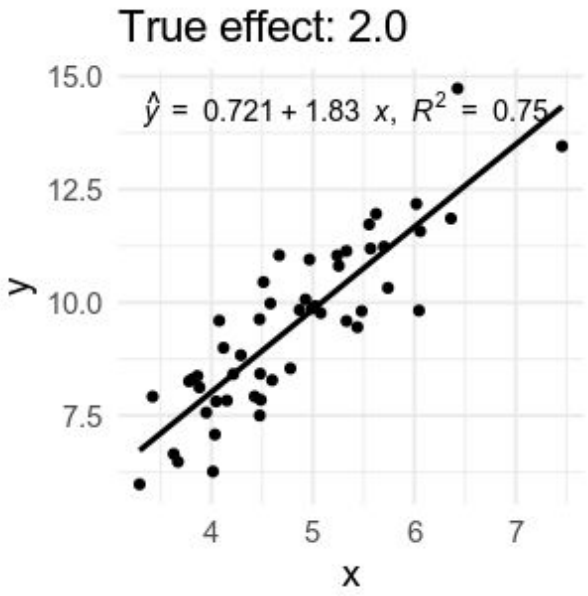
$$y = f(x) + \epsilon$$

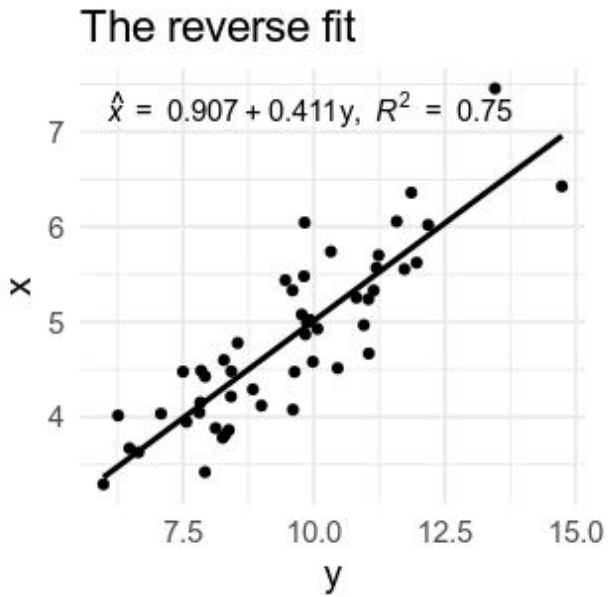
Statistical and machine-learning models

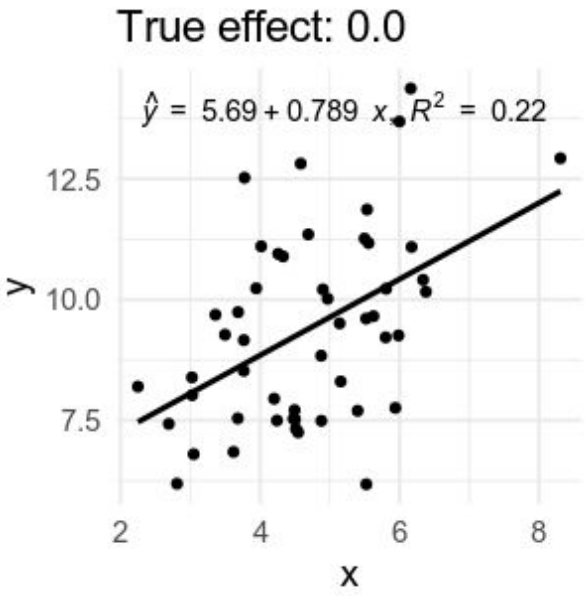


Causal models

Correlation is caused by causation, confounding, coincidence, or conspiracy







Statistical models alone cannot derive causality from correlation

We learn causality by (1) listing models explicitly and (2) manipulating a variable and observe the outcomes

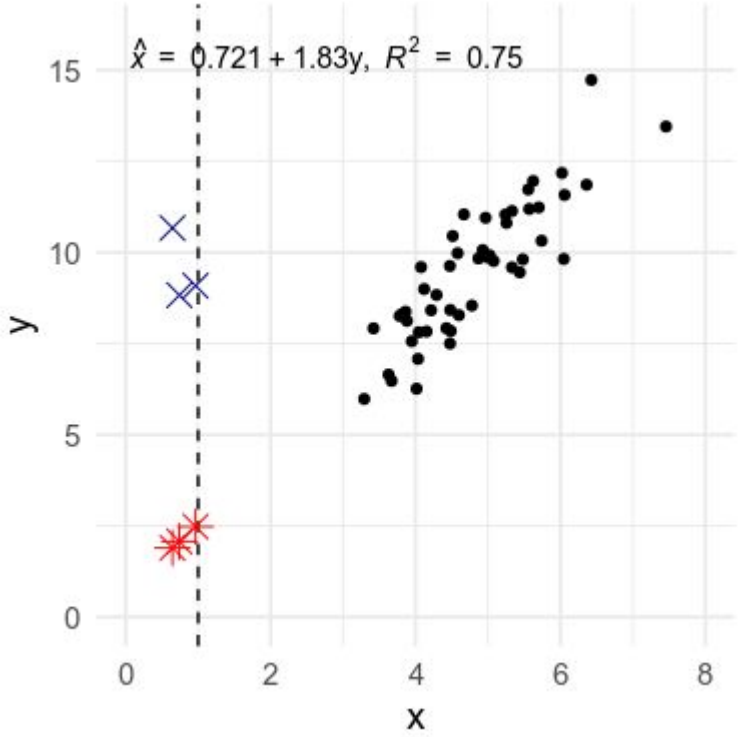
Model 1

Model 2

Model 3

Assume that the data is generated by either Model 1, or Model 2, or Model 3. And assume that we can manipulate the value of X by setting it to 1.0 (the dash line).

Question: which outcomes (red stars or blue crosses) would support which models? Why?



Causality is crucial for drug discovery

?

?

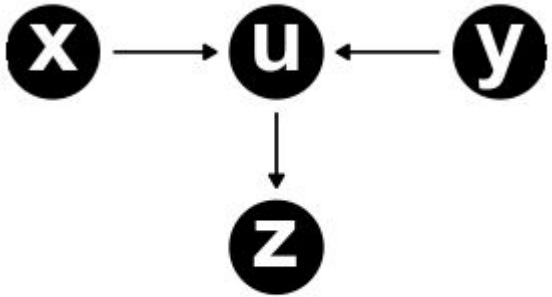
Biomarker, tox study, pathology, omics data, real-world data, …

	x	z	y
1	0.835386320	1	-0.73897252
2	-0.005354014	-1	-0.82972315
3	0.058788286	1	0.76213369
4	-1.015602246	-1	-0.05951719
5	-0.339569780	-1	-0.11745910
6	-0.041077979	-1	-1.28243716
7	0.363740407	1	-0.30570762
8	0.119496314	-1	-1.19932461
9	0.257108454	-1	-1.06044066
10	0.304537158	-1	-0.43396492

?

?

The descendant



We need both models (knowledge + assumptions) and data to infer causality.

Accurate predictions on small data with a tabular foundation model

Almost too good to be true?

"I knew the indoor pool was too good to be true."

Tabular Prior-data Fitted Network (TabPFN)

Article

Accurate predictions on small data with a tabular foundation model

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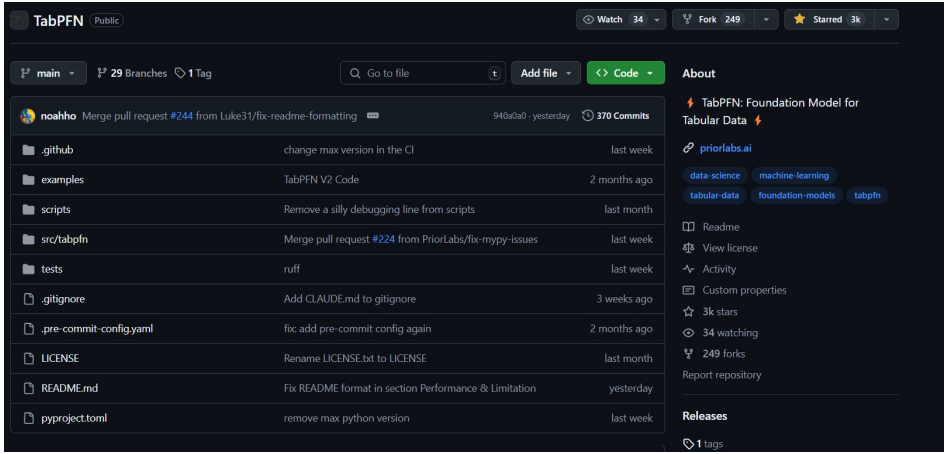
Noah Hollmann[1,2,3,7], Samuel Müller[1,7], Lennart Purucker[1], Arjun Krishnakumar[1], Max Körfer[1], Shi Bin Hoo[1], Robin Tibor Schirrmeister[4,5] & Frank Hutter[1,3,6]

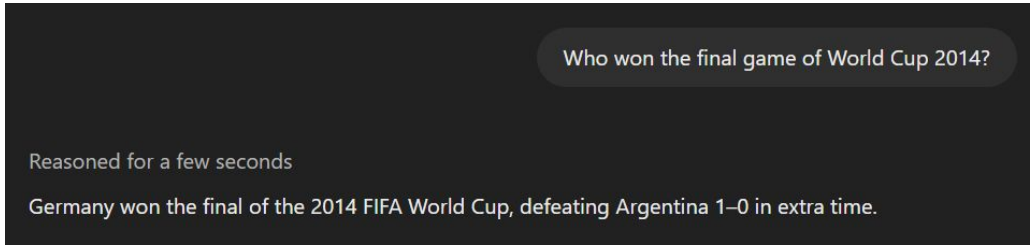
Tabular data, spreadsheets organized in rows and columns, are ubiquitous across scientific fields, from biomedicine to particle physics to economics and climate science[1,2]. The fundamental prediction task of filling in missing values of a label column based on the rest of the columns is essential for various applications as diverse as biomedical risk models, drug discovery and materials science. Although deep learning has revolutionized learning from raw data and led to numerous high-profile success stories[3–5], gradient-boosted decision trees[6–9] have dominated tabular data for the past 20 years. Here we present the Tabular Prior-data Fitted Network (TabPFN), a tabular foundation model that outperforms all previous methods on datasets with up to 10,000 samples by a wide margin, using substantially less training time. In 2.8 s, TabPFN outperforms an ensemble of the strongest baselines tuned for 4 h in a classification setting. As a generative transformer-based foundation model, this model also allows fine-tuning, data generation, density estimation and learning reusable embeddings. TabPFN is a learning algorithm that is itself learned across millions of synthetic datasets, demonstrating the power of this approach for algorithm development. By improving modelling abilities across diverse fields, TabPFN has the potential to accelerate scientific discovery and enhance important decision-making in various domains.



Source code at https://github.com/PriorLabs/tabpfn, released in a license analogous to Apache 2.0 (commercial use friendly).

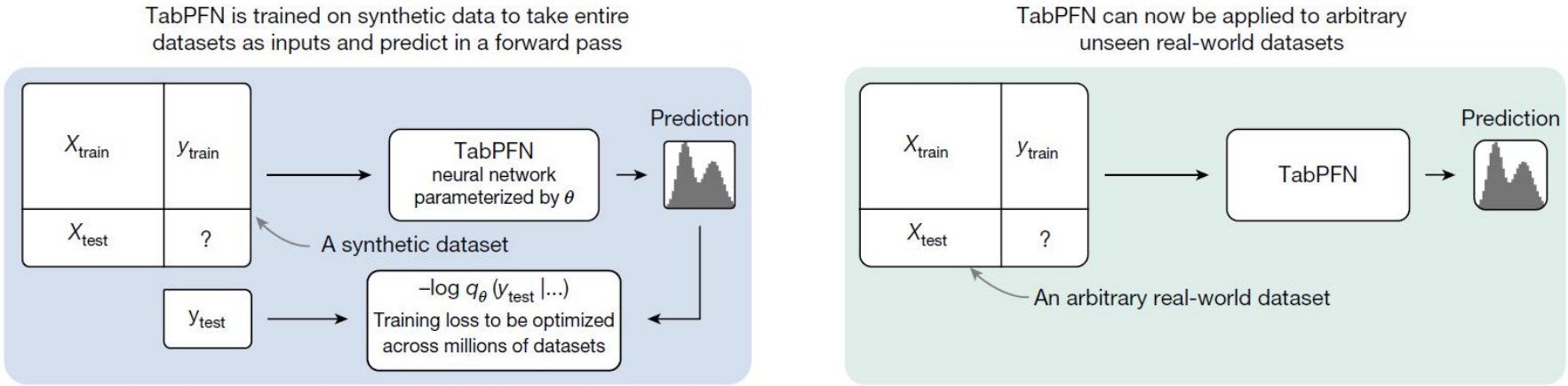
In-Context Learning (ICL)

The model isn't explicitly trained on a particular task ahead of time. Instead, the prompt itself provides examples that enable the model to perform the task on the fly. It is one of the intriguing abilities of deep transformer models.



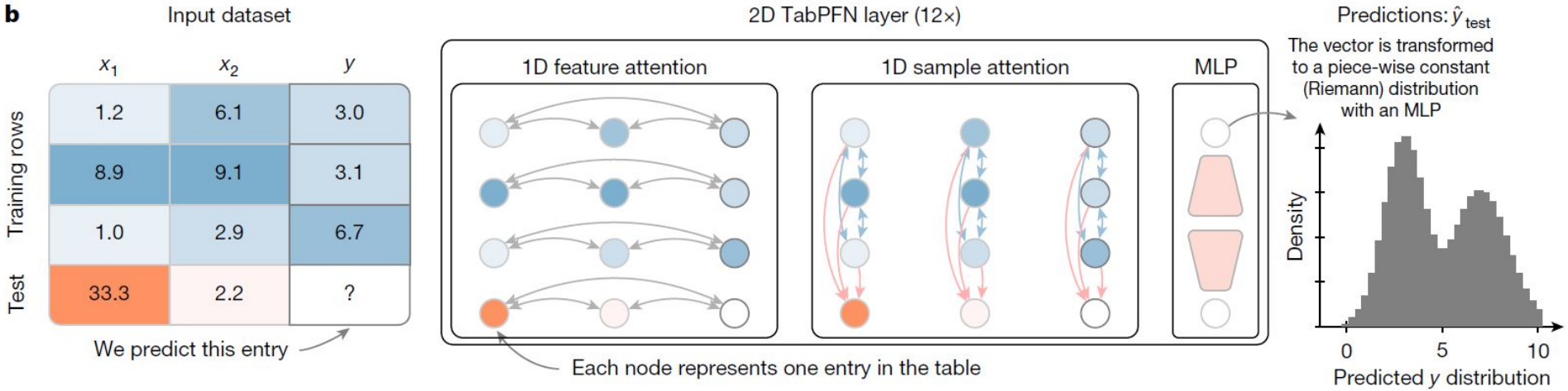
Left: chat history (ChatGPT o1 model, tested on 18.03.2025). Right: The ground truth.

Tabular Prior-data Fitted Network (TabPFN) is trained with synthesized data and predicts missing value in user data

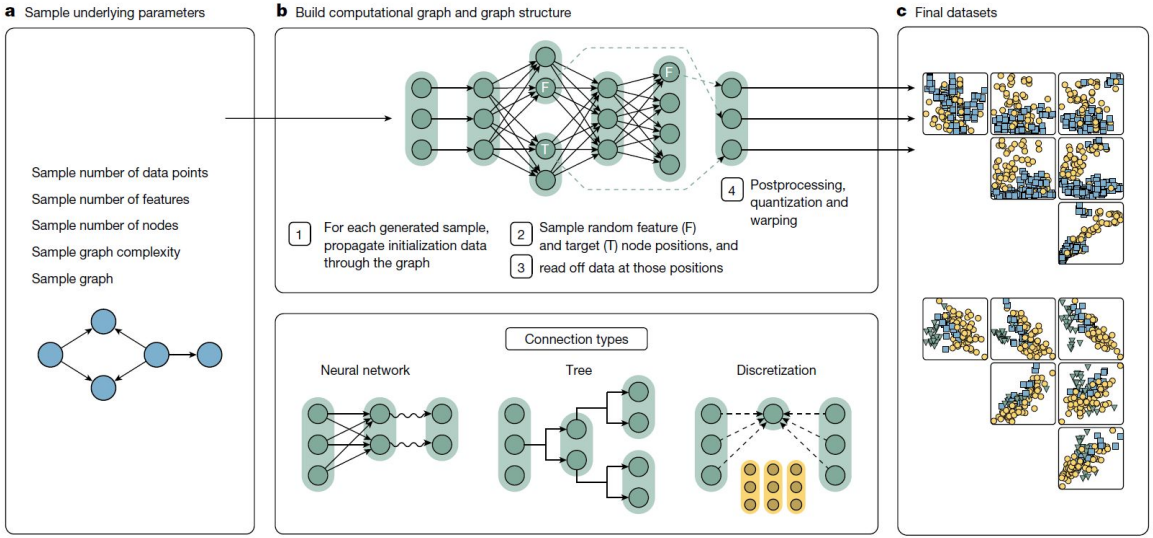


A metaphor: Imagine a lab where millions of billiard games are played simultaneously: in each game, different numbers of balls are placed randomly, and a white ball starts with a random velocity at a random position. By learning the trajectory of all balls of all games, one may learn to predict the trajectory of any real billiard game, as long as the positions of all balls and the initial velocity of the white ball is known.

Architecture of TabPFN uses both row-wise sample and column-wise feature information to predict a distribution

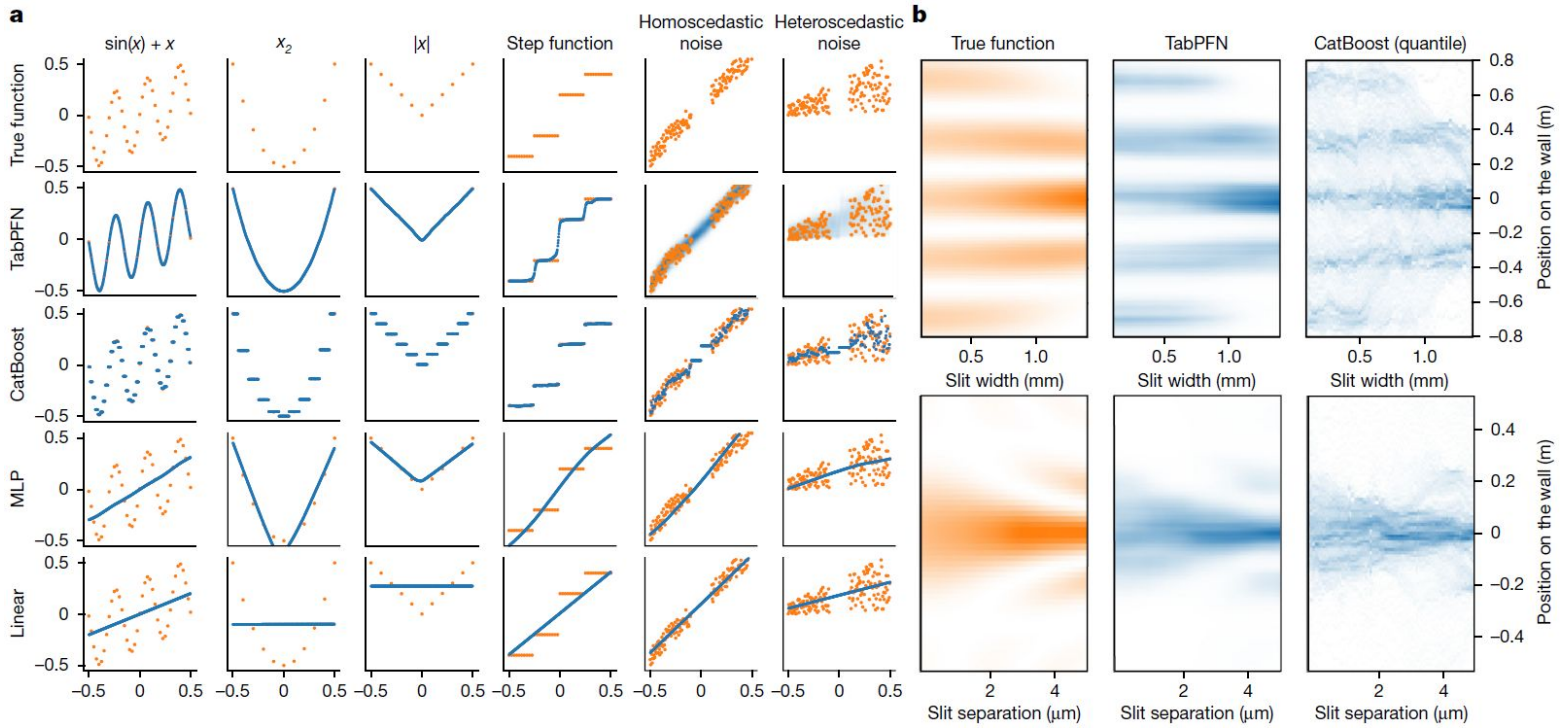


Generating synthesized data with causal models

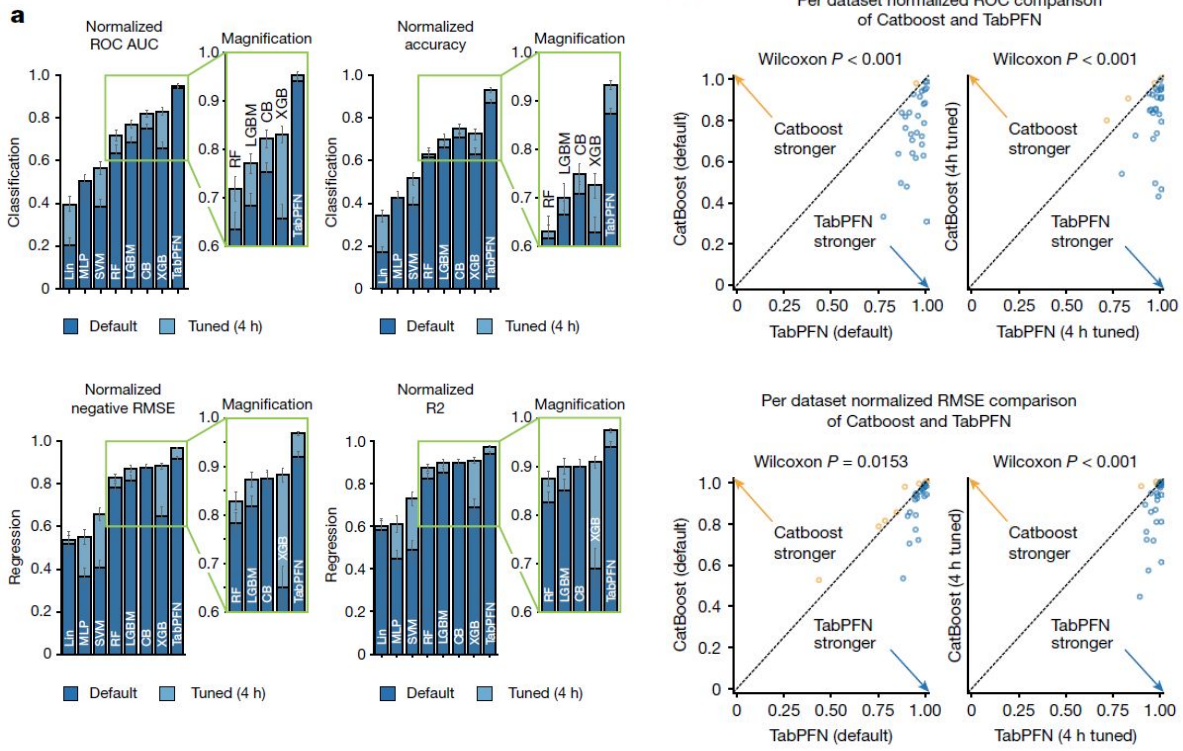


1. We sample 100-million structurally unique directed acyclic graphs (DAGs) as causal graphs that generate data.
2. Random data is assigned to the root node. Other nodes are propagated by rules specified by the edges, plus a Gaussian noise.
3. The values of sampled features (input to the model) and targets (output of the model) are extracted.
4. Data are post-processed (non-linear distortion, binning, random missing) to reflect real-world data processing.
5. Synthesized data from 10^8 causal experiments are used to train *TabPFN*.

TabPFN's prediction on data generated by simple functions



Performance benchmark against popular methods



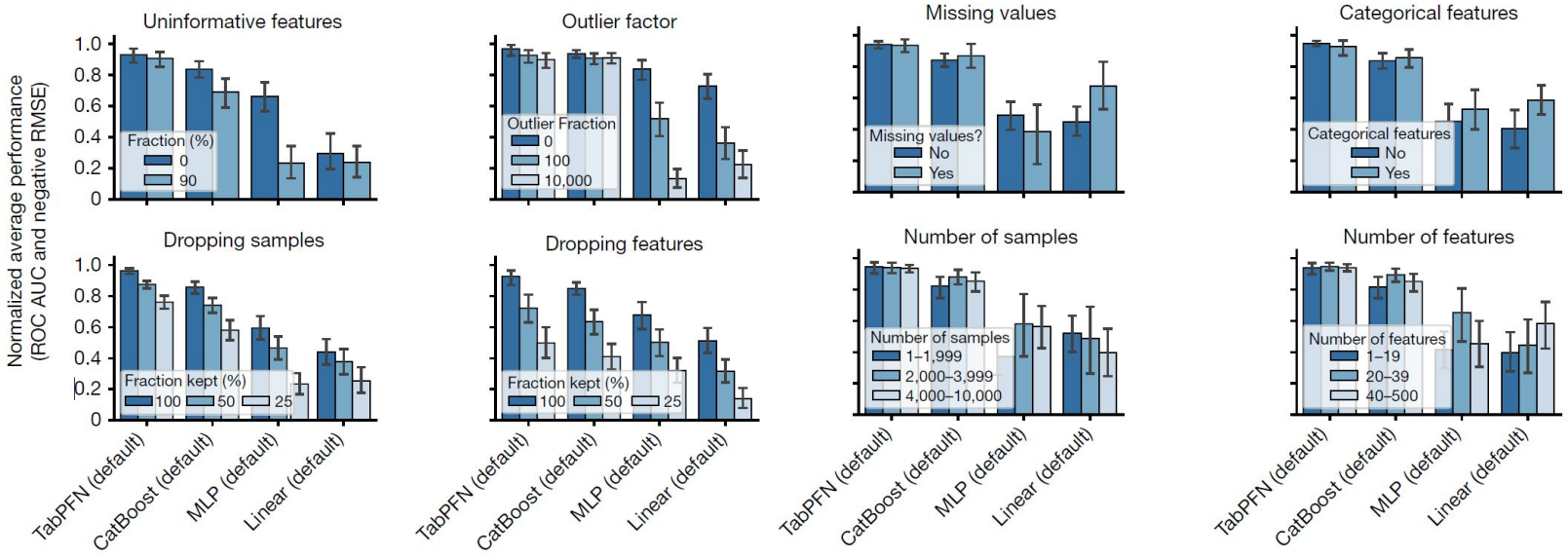
Left: Performance for classification (top) and regression (bottom) tasks.

Right: Comparing performance of TabPFN and CatBoost, the top contendant, for classification (top) and regression (bottom) tasks.

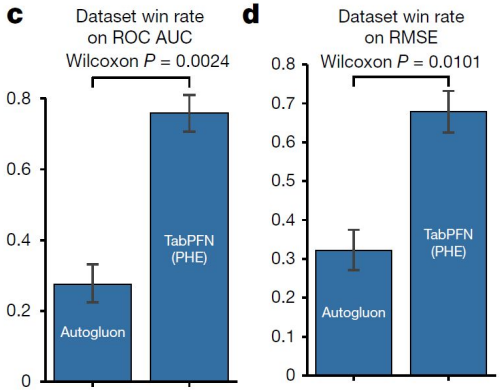
Hollmann, Noah, Samuel Müller, …, Frank Hutter. 2025. “Accurate Predictions on Small Data with a Tabular Foundation Model.” Nature 637 (8045): 319–26.

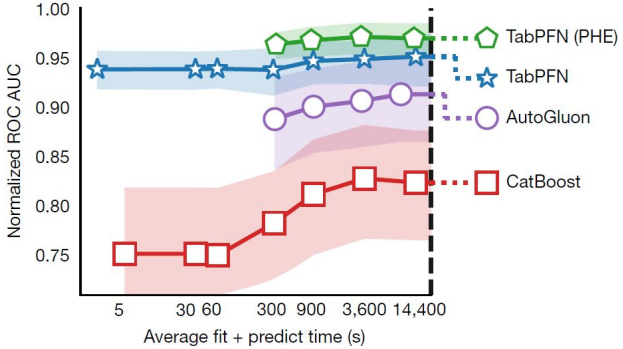
Lin: linear; MLP: multi-layer perceptrons; SVM: support vector machines; RF: random forest; LGBM: Light Gradient Boosting Machine; CB: CatBoost; XGB: XGBoost; ROC: receiver operating

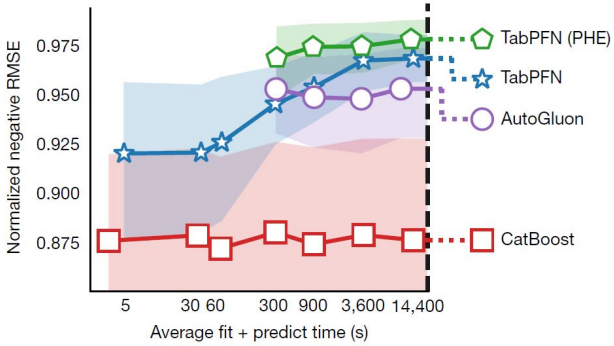
Robustness against common caveats and problems



Benchmark against AutoML and CatBoost







Datasets that the authors tested for classification (left) and regression (right) tasks

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Extended Data Table 3 | List of test datasets used for primary evaluation of classification tasks

Name	OpenML ID	Domain	Features	Samples	Targets	Categorical Feats.
ada	41156	Census	48	4147	2	0
Australian	40981	Finance	14	690	2	8
blood-transfusion-service-center	1464	Healthcare	4	748	2	0
car	40975	Automotive	6	1728	4	6
churn	40701	Telecommunication	20	5000	2	4
cmc	23	Public Health	9	1473	3	7
credit-g	31	Finance	20	1000	2	13
dna	40670	Biology	180	3186	3	180
eucalyptus	188	Agriculture	19	736	5	5
first-order-theorem-proving	1475	Computational Logic	51	6118	6	0
GesturePhase Segmentation Processed	4538	Human-Computer Interaction	32	9873	5	0
jasmine	41143	Natural Language Processing	144	2984	2	136
kc1	1067	Software Engineering	21	2109	2	0
kr-vs-kp	3	Game Strategy	36	3196	2	36
madeline	41144	Artificial	259	3140	2	0
mfeat-factors	12	Handwriting Recognition	216	2000	10	0
ozone-level-8hr	1487	Environmental	72	2534	2	0
pc4	1049	Software Engineering	37	1458	2	0
philippine	41145	Bioinformatics	308	5832	2	0
phoneme	1489	Audio	5	5404	2	0
qsar-biodeg	1494	Environmental	41	1055	2	0
Satellite	40900	Environmental Science	36	5100	2	0
segment	40984	Computer Vision	16	2310	7	0
steel-plates-fault	40982	Industrial	27	1941	7	0
sylvine	41146	Environmental Science	20	5124	2	0
vehicle	54	Image Classification	18	846	4	0
wilt	40983	Environmental	5	4839	2	0
wine-quality-white	40498	Food and Beverage	11	4898	7	0
yeast	181	Biology	8	1484	10	0

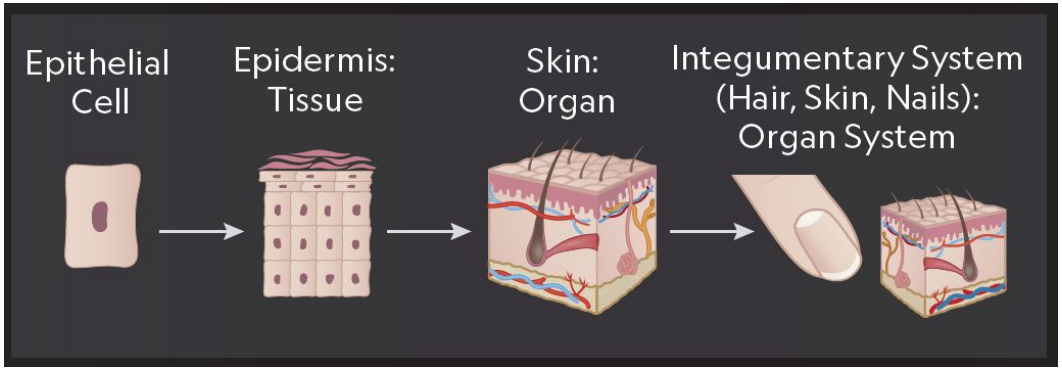
All classification tasks from the AutoML Benchmark[36] with fewer 10,000 samples and 500 features. The benchmark comprises diverse real-world tabular datasets, curated for complexity, relevance, and domain diversity.

Extended Data Table 4 | List of test datasets used for primary evaluation of regression tasks

Name	OpenML ID	Domain	Features	Samples	Categorical Features
abalone	42726	Marine Biology	8	4177	1
airfoil_self_noise	44957	Aerospace Engineering	5	1503	0
auction_verification	44958	Economics	7	2043	2
boston	531	Real Estate	13	506	2
cars	44994	Automotive Engineering	17	804	0
colleges	42727	Education	44	7063	12
concrete_compressive_ strength	44959	Materials Science	8	1030	0
cpu_activity	44978	Computer Engineering	21	8192	0
energy_efficiency	44960	Architectural Engineering	8	768	0
geographical_origin _of_music	44965	Music Information Retrieval	116	1059	0
grid_stability	44973	Power Systems Engineering	12	10000	0
house_prices_nominal	42563	Real Estate	79	1460	43
kin8nm	44980	Robotics	8	8192	0
Mercedes_Benz_ Greener_ Manufacturing	42570	Manufacturing	376	4209	8
MIP-2016-regression	43071	Operations Research	144	1090	1
Moneyball	41021	Sports Analytics	14	1232	6
pumadyn32nh	44981	Robotics	32	8192	0
QSAR_fish_toxicity	44970	Toxicology	6	908	0
quake	550	Geophysics	3	2178	0
SAT11-HAND-runtime-regression	41980	Computational Logic	116	4440	1
sensory	546	Food Science	11	576	11
socmob	541	Sociology	5	1156	4
space_ga	507	Political Science	6	3107	0
student_performance	44967	Education	30	649	17
tecator	505	Food Science	124	240	0
topo_2_1	422	Cheminformatics	266	8885	0
us_crime	42730	Criminology	126	1994	0
yprop_4_1	416	Cheminformatics	251	8885	0

All regression tasks from the AutoML[36] and OpenML-CTR23[37] Benchmarks with fewer 10,000 samples and 500 features. The benchmark comprises diverse real-world tabular datasets, curated for complexity, relevance, and domain diversity.

Complexity Increases Through a System

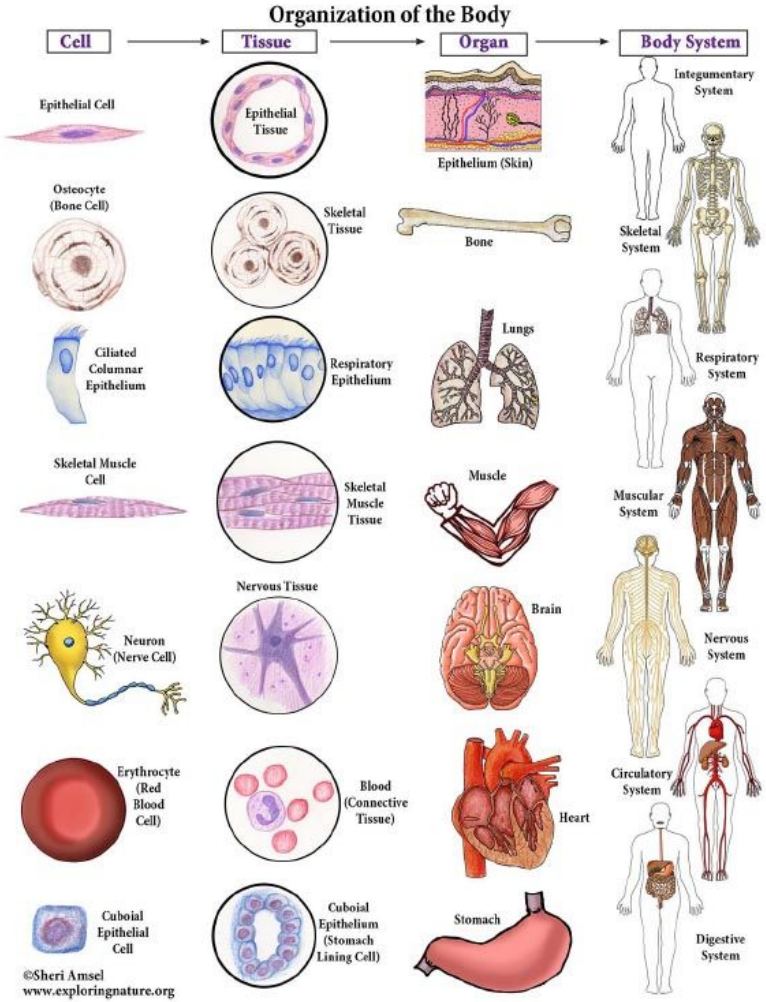


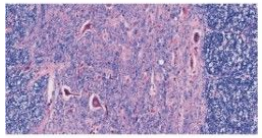
Cells: basic building blocks, variable morphologies and functions

Tissues: groups of specialized cells that communicate and collaborate

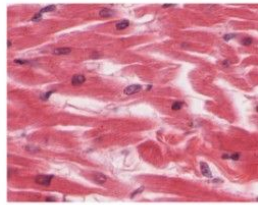
Organ: group of tissues to perform specific functions

Organ systems: group of organs and tissues



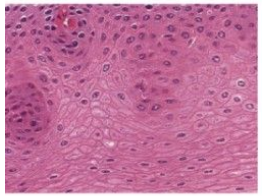


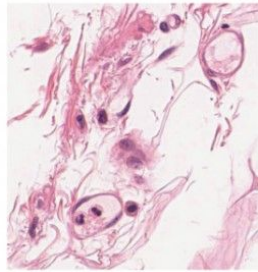
Nervous tissue
Brain
Spinal cord
Nerves



Muscle tissue
Cardiac muscle
Smooth muscle
Skeletal muscle

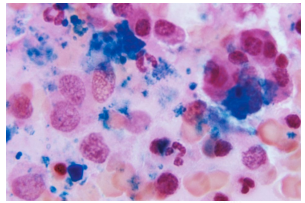
Epithelial tissue
Lining of GI tract organs and other hollow organs
Skin surface (epidermis)

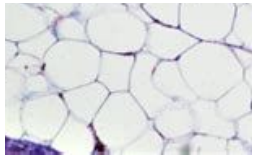


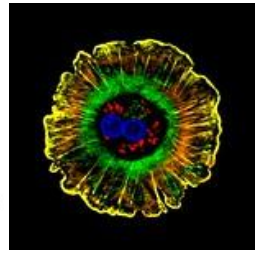


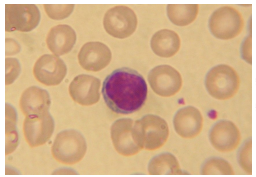
Connective tissue
Fat and other soft padding tissue
Bone
Tendon

Four major tissue types







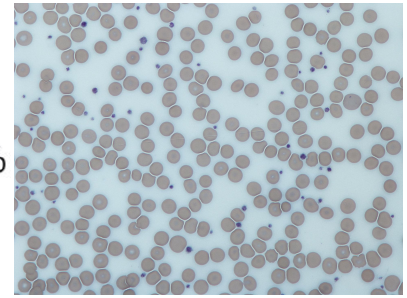


adipocytes 0.2%

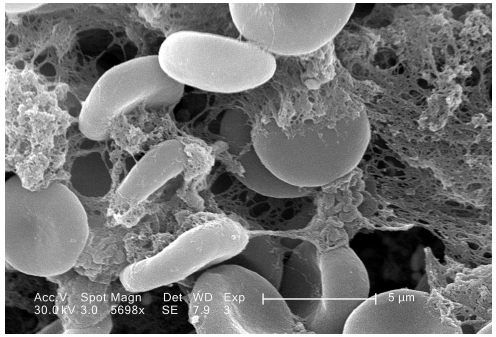
lymphocytes 1.5%

bone marrow cells 2.5%

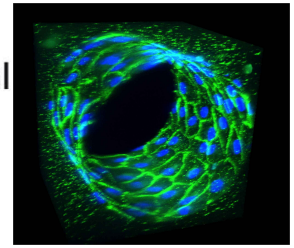
hepatocytes 0.8%



platelets 4.9%

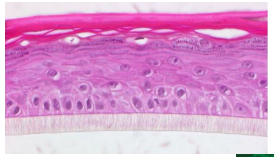


erythrocytes, 84%
25×10^{12} of 30×10^{12}
total human cell count



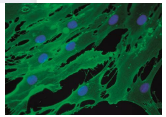
bronchial endothelial cells 0.5%

vascular endothelial cells 2.1%



epidermal cells 0.5%

respiratory interstitial cells 0.5%

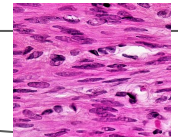


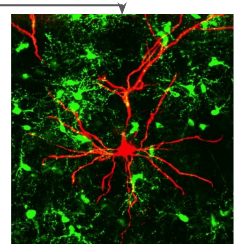
dermal fibroblasts 0.1%

muscle cells 0.001%

other 2.0%

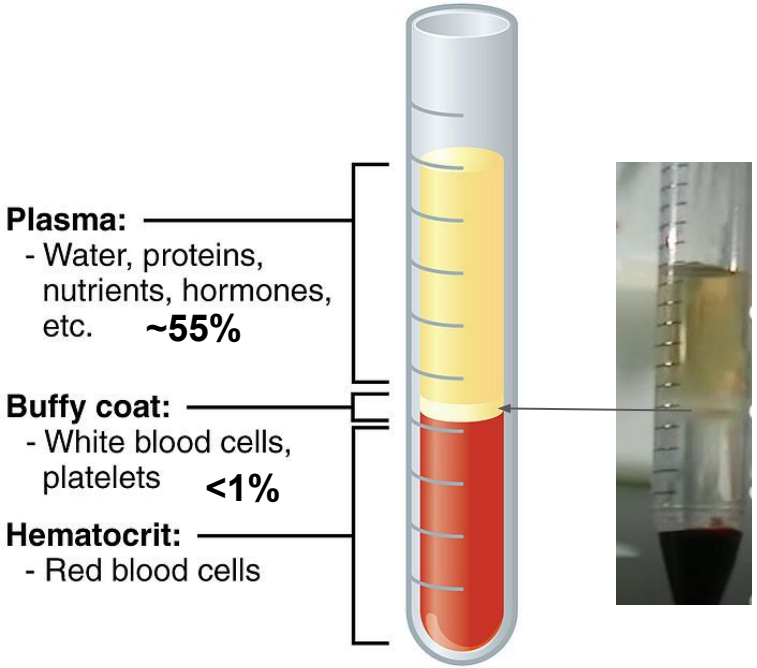
neurons and glia 0.6%

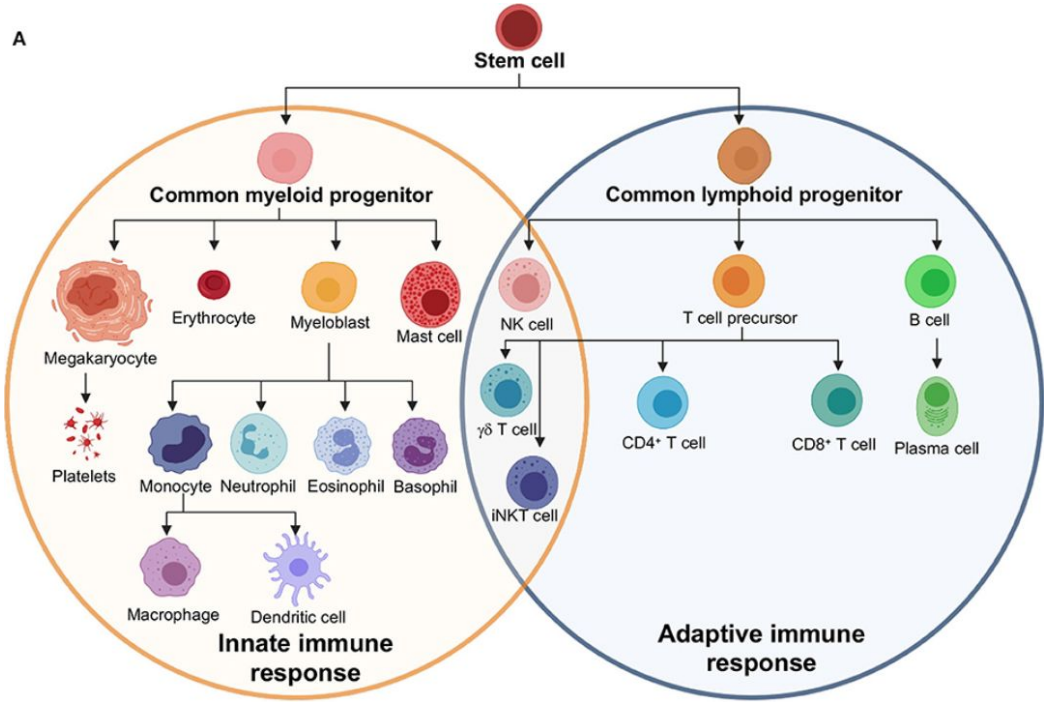




UNI BASEL

What's in a drop of blood? Ask a doctor or a biologist!

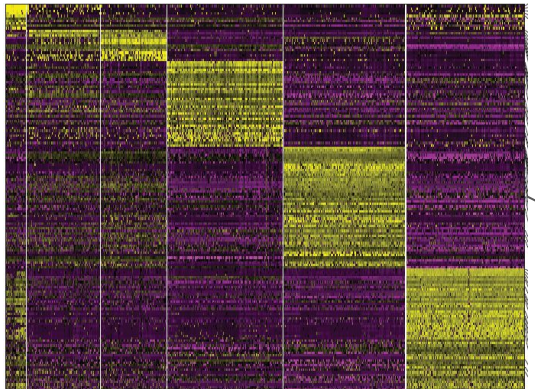




What's in a drop of blood? Count the genes!

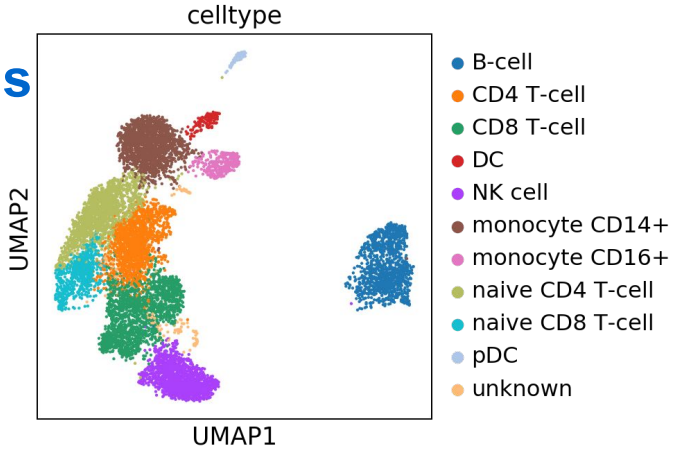
Sequencing

Genes



Cells

Data analysis



Low Expression

High Expression

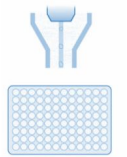
Single-cell sequencing (scSeq) workflow

Tissue dissociation

Single cell capture and transcriptome sequencing

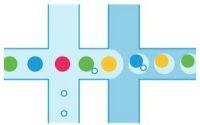
Solid tissue

Dissociated cells/nuclei



Flow cytometry 96-384 cells

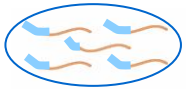
Cell/nuclei suspension



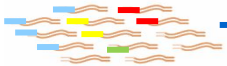
Droplet microfluidics 1000s of cells

Throughput

Single cells or nuclei



cDNA

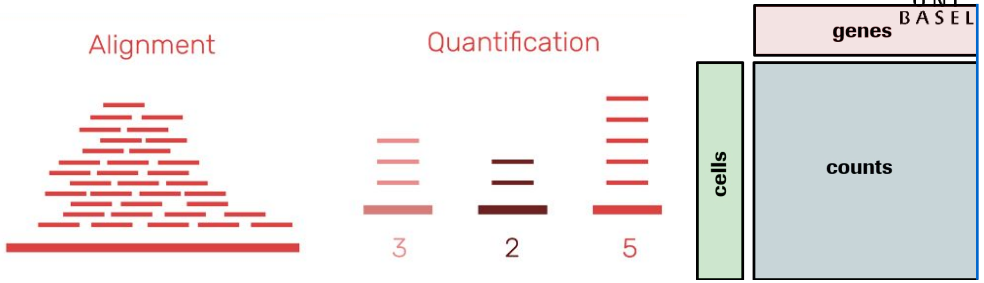


Pooled cDNA libraries

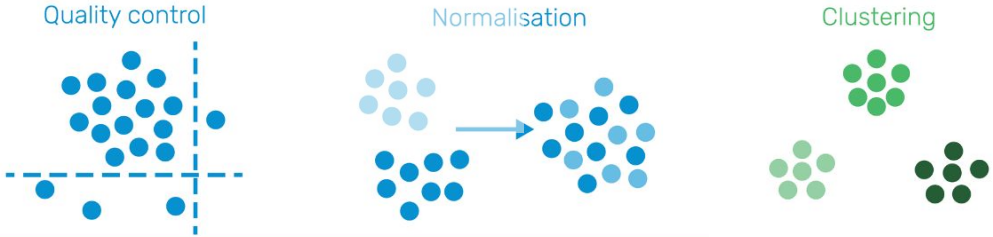
Next-Generation Sequencing

A linearized workflow of scSeq data analysis

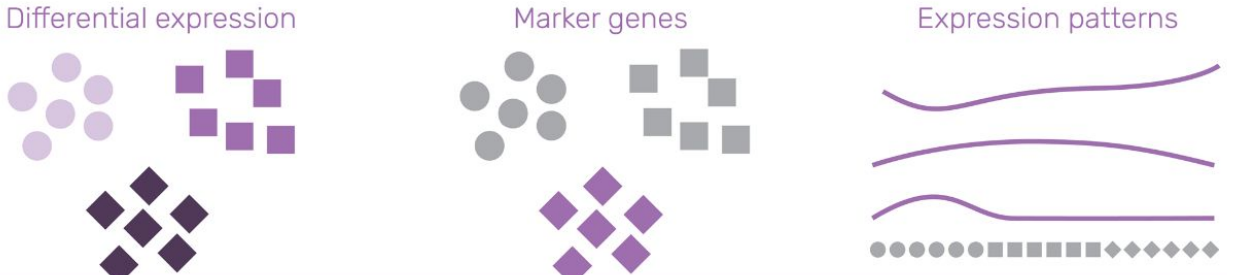
From short reads to gene-cell matrix



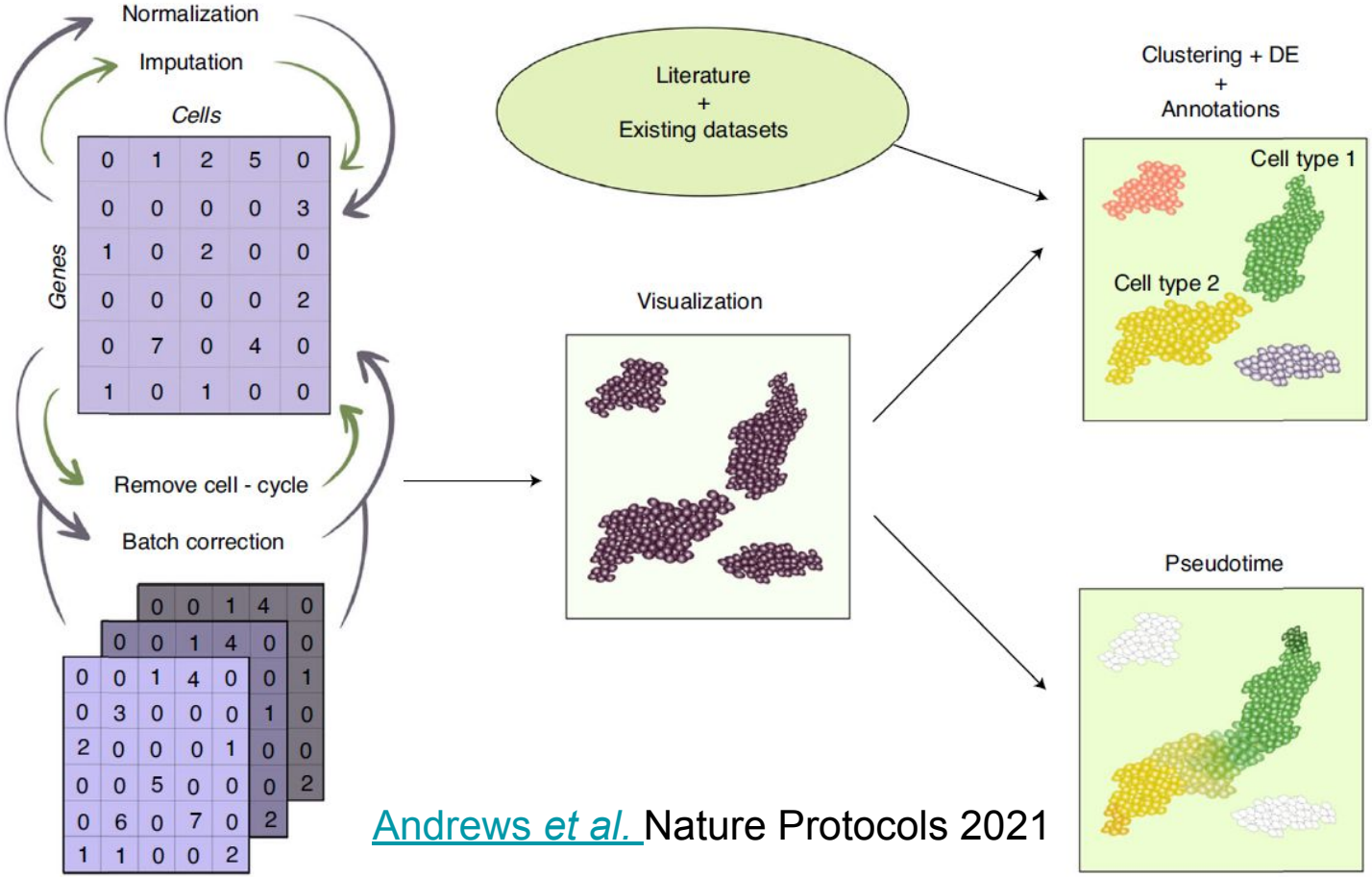
QC, filtering & normalization, dimensionality reduction, and clustering



Downstream analysis

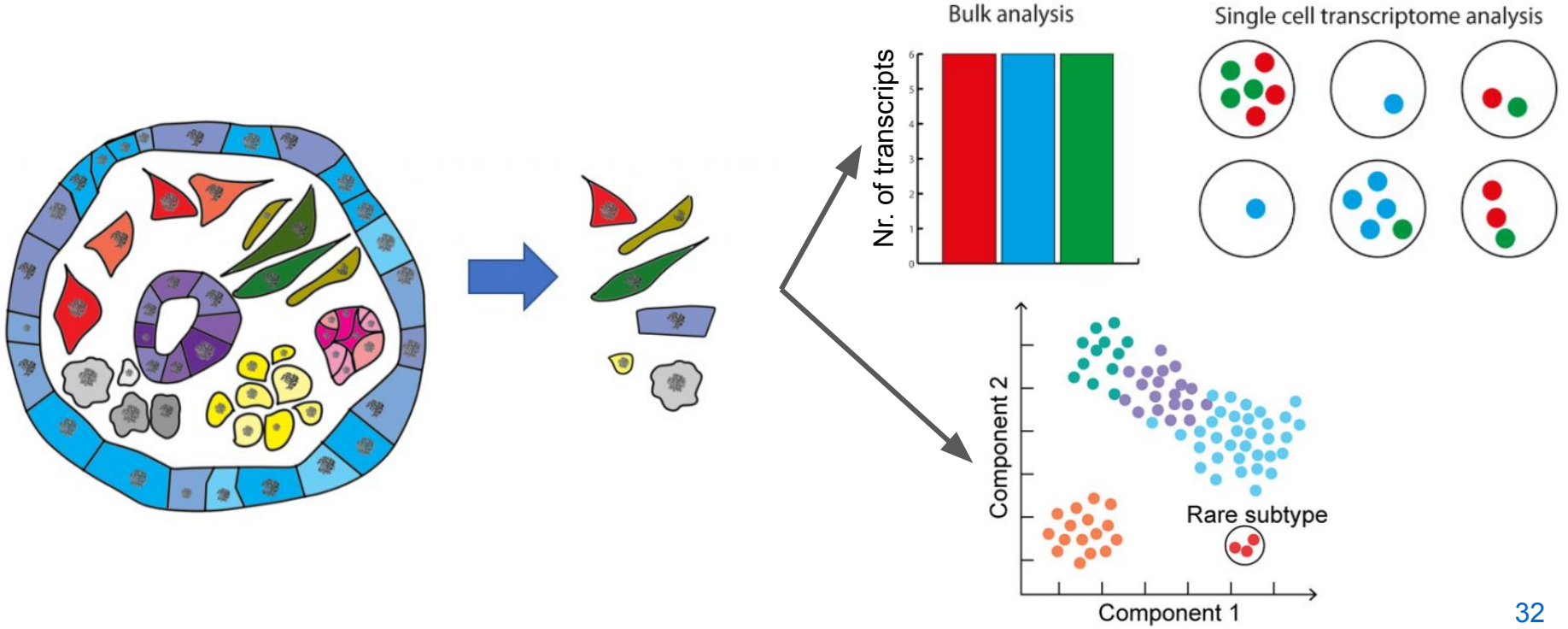


Overview of the computational workflow



Andrews *et al.* Nature Protocols 2021

Single-cell biology benefits both disease understanding and drug discovery

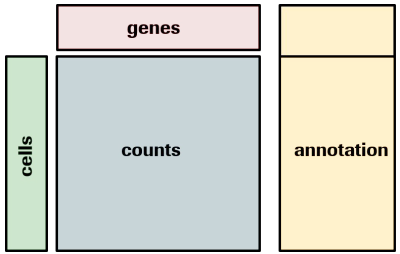


BESCA: An open-source Python package for single-cell gene expression analysis

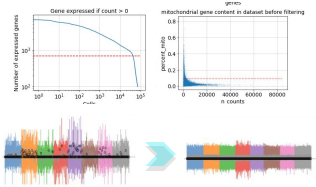
An automatized standard workflow

Data loading | Preprocessing | Clustering | Visualization | Analysis

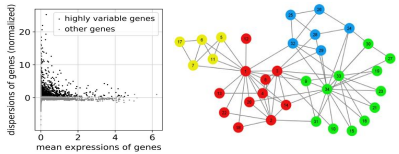
From FAIR format



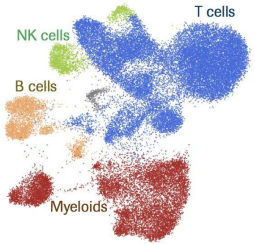
Quality Control
Filtering,
Normalization



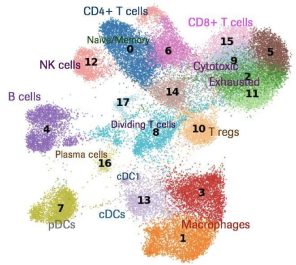
Dimensionality
reduction
Clustering



UMAP



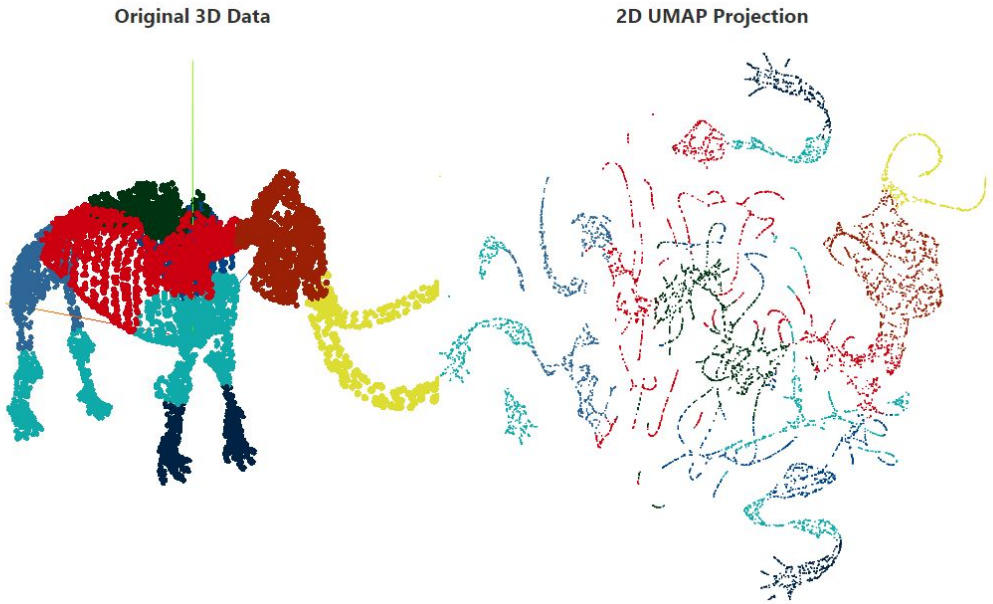
Cell type
characterisation

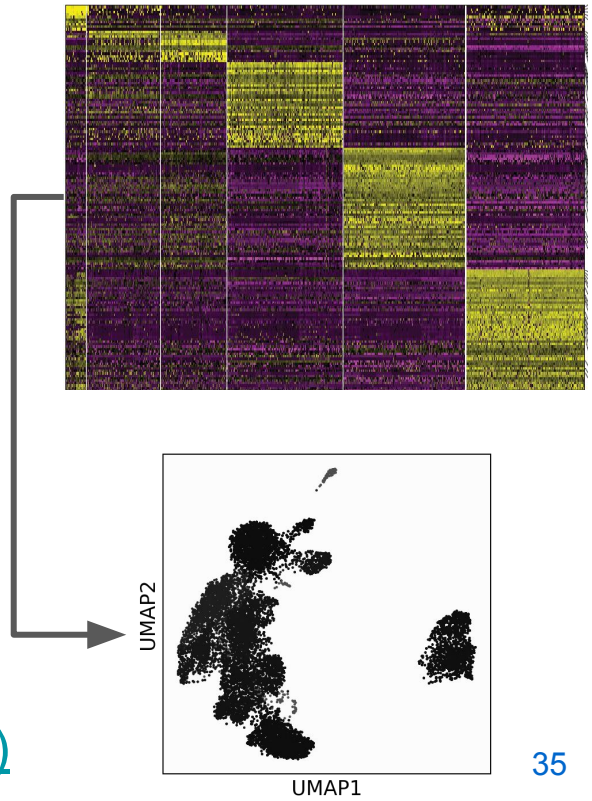


How to represent voxels with pixels?

The elephant bull *Tusker (1992-2023)* at Zolli Basel plays with a tree trunk on a post (2022)

Uniform Manifold Approximation and Projection (UMAP) for dimension reduction

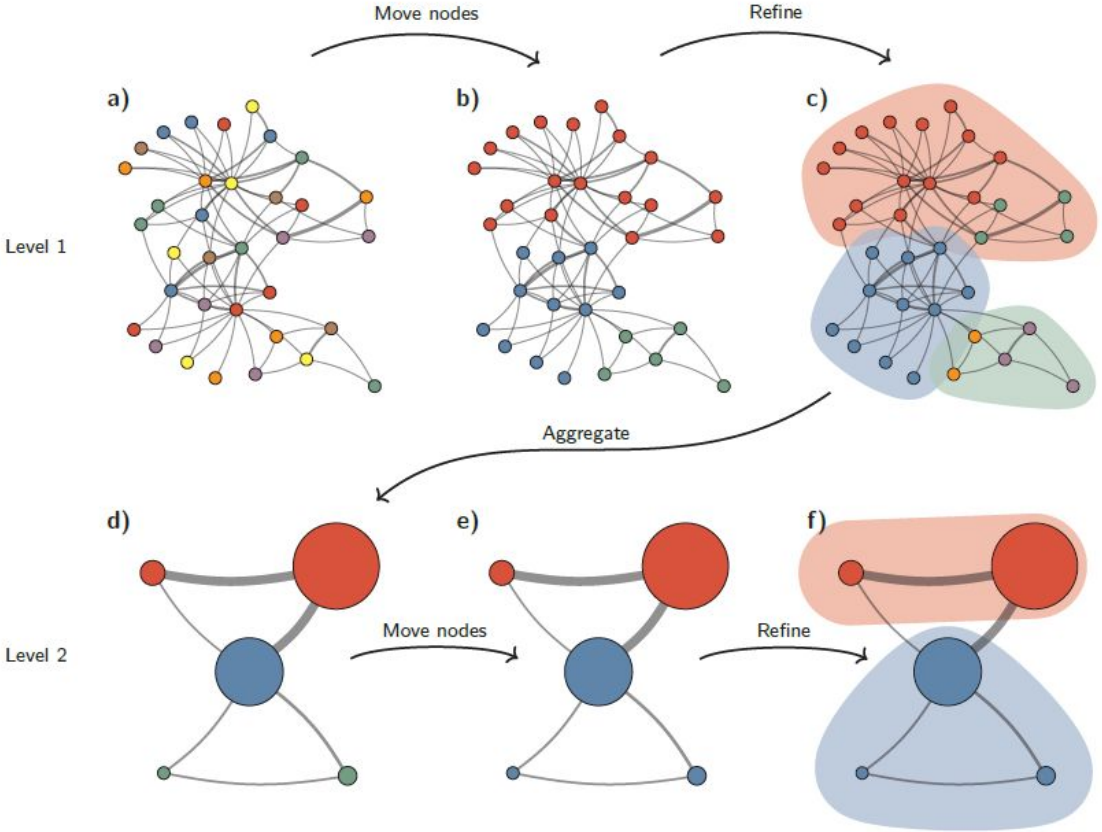


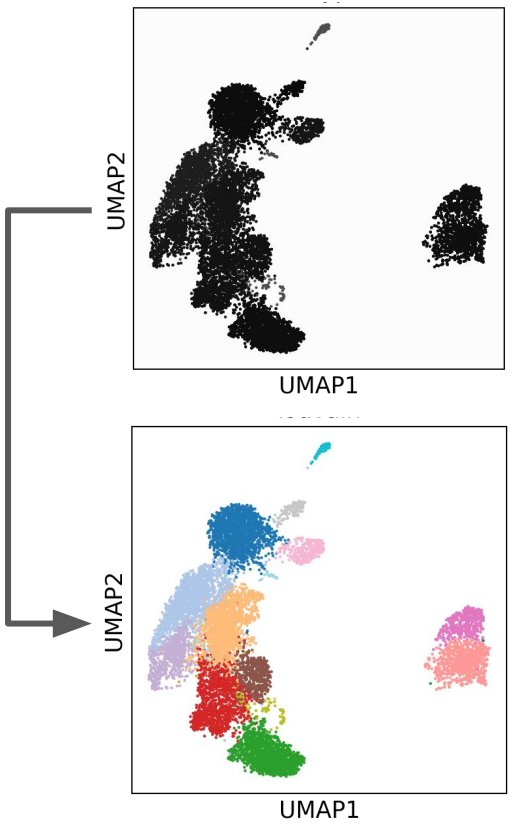


Understanding UMAP by A. Coenen and A. Pearce

UMAP by Leland McInnes on SciPy 2018 (YouTube)

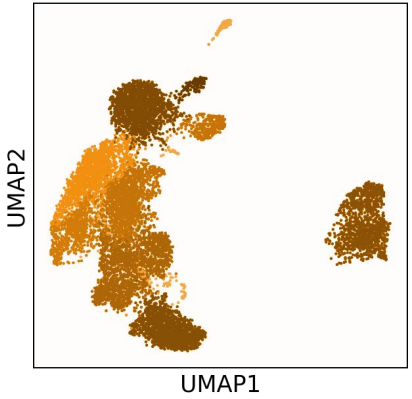
The Leiden Algorithm for Community Detection



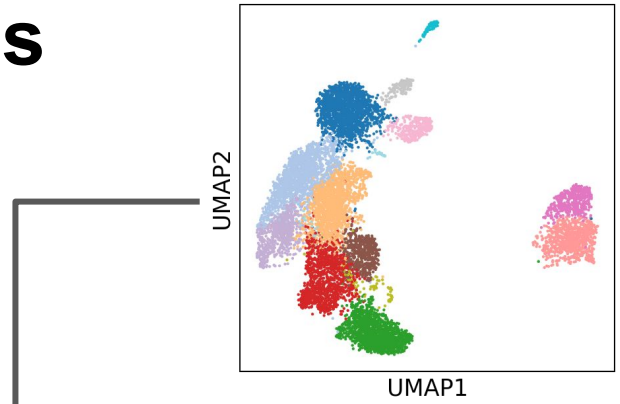


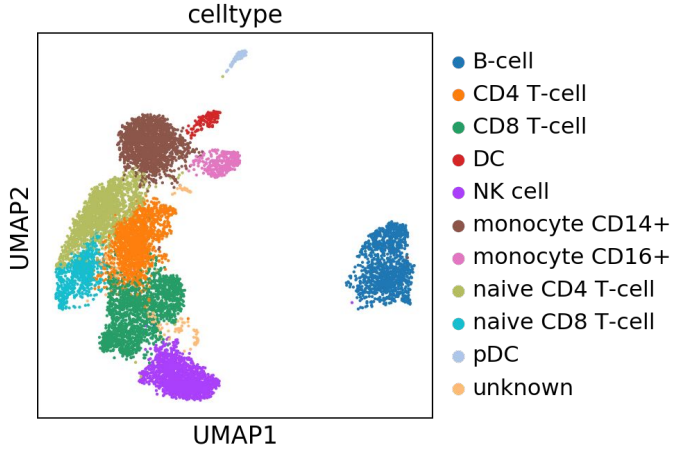
Biological knowledge and visual inspection is used to annotate cell types

Heatmap of gene X

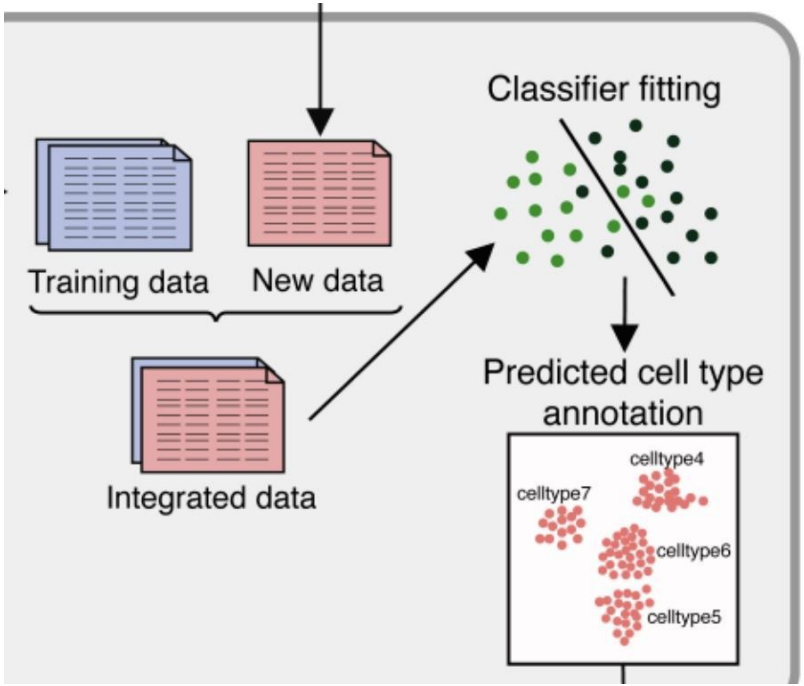


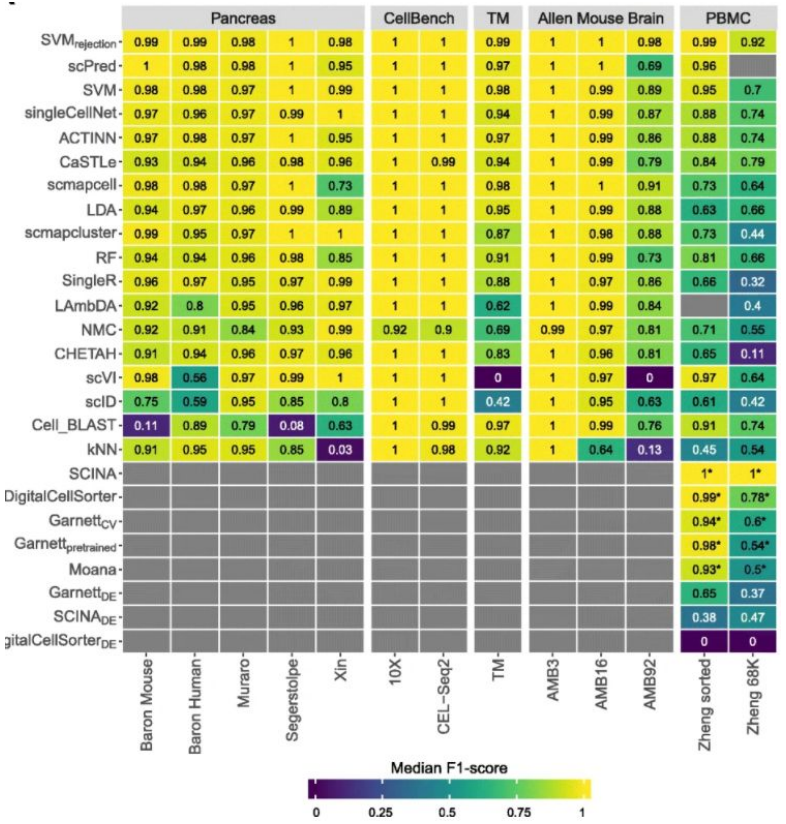
lymphocyte	PTPRC							
myeloid	S100A8	S100A9	CST3					
Bcell	CD19	CD79A	MS4A1					
Tcells	CD3E	CD3G	CD3D					
CD4	CD4							
CD8	CD8A	CD8B						
NKcell	NKG7	GNLY	NCAM1					
monocyte	CST3	CSF1R	ITGAM	CD14	FCGR3A	FCGR3B		
macrophage	CD14	IL1B	LYZ	CD163	ITGAX	CD68	CSF1R	FCGR3A



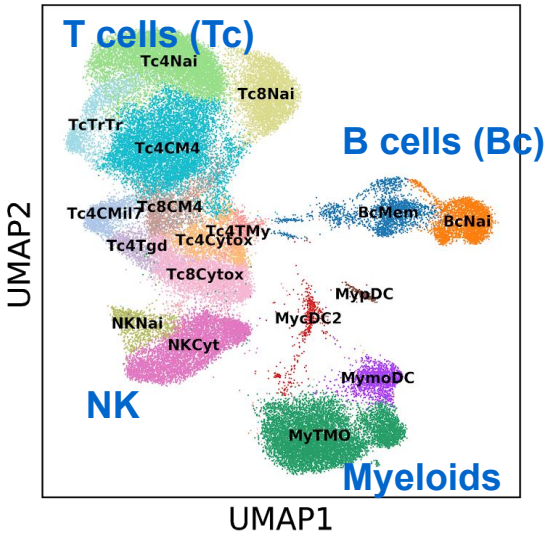


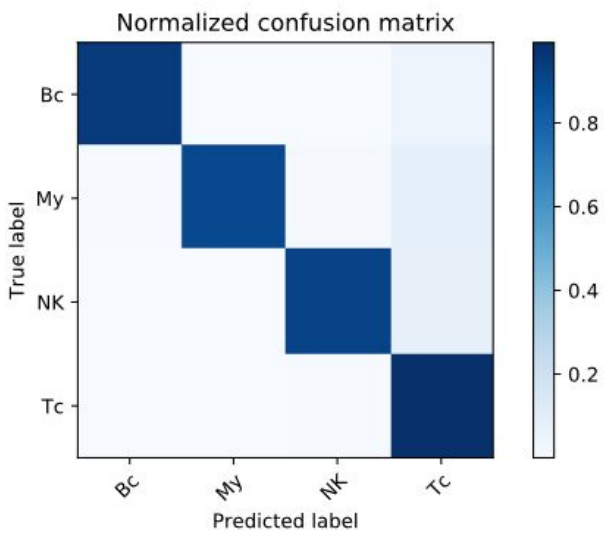
Cell type annotation with machine learning

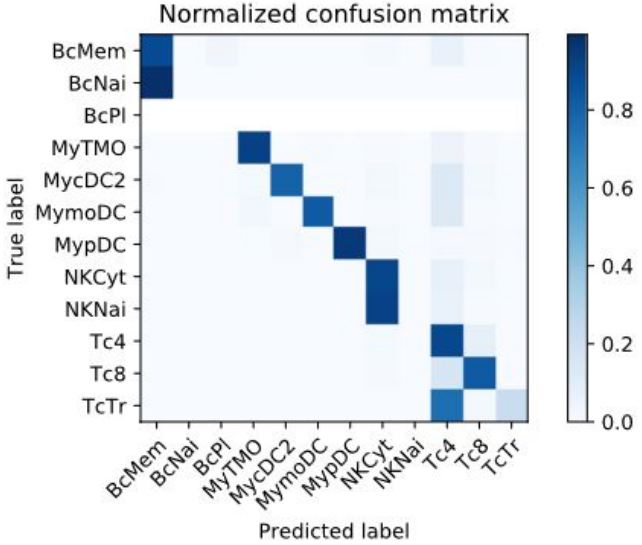




A PBMC example of cell type annotation







- Broad level cell types, including B cells (Bc), Myeloid (My), NK cells (NK) and T cells (Tc), are successfully predicted.
- Missing and highly similar cell types cause challenges with increased granularity. Essential: reference data quality and knowledge of cell types.

Single-cell biology is important in drug discovery

Disease understanding: disease-specific cell types and states

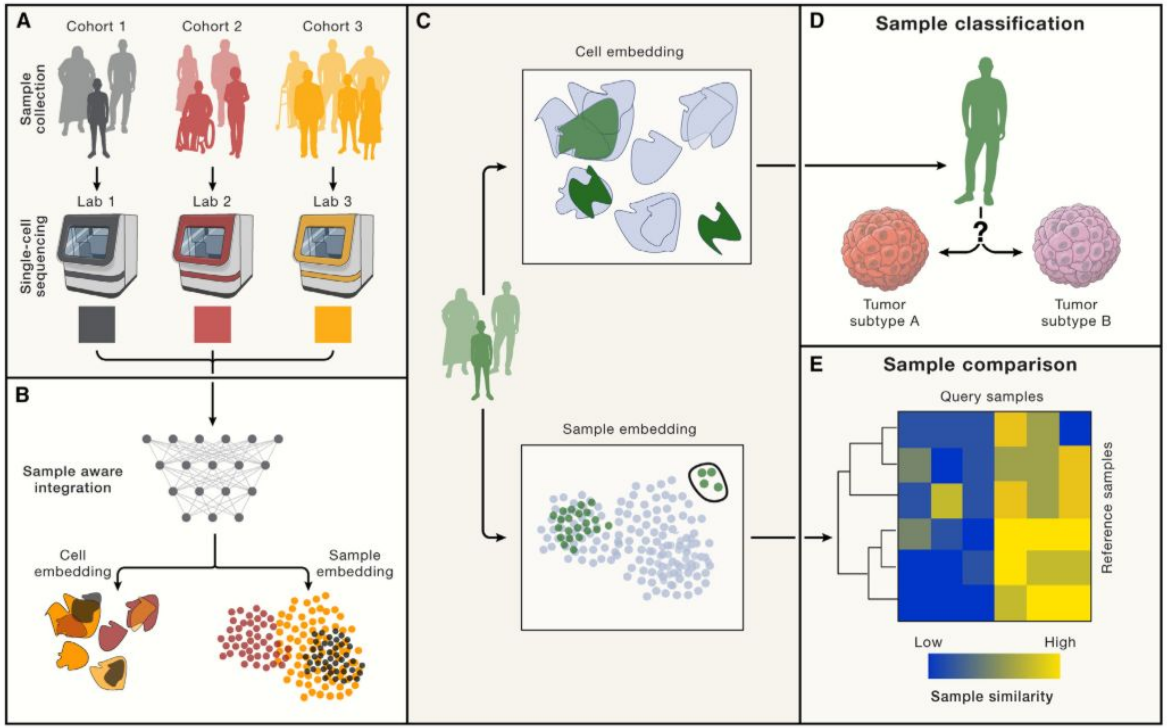
Biomarker and patient stratification: which genes should we measure in which cell type(s)?

Target identification: expression pattern in health and disease across cell types

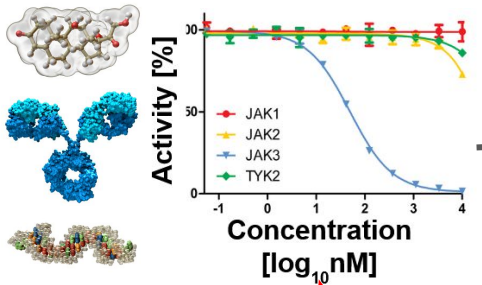
MoA and safety modelling: perturbation effect at single-cell level

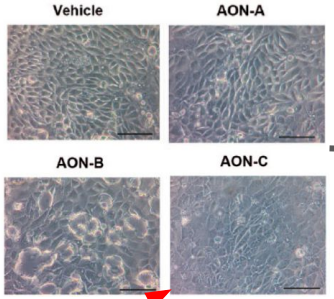
Reference mapping at population scale

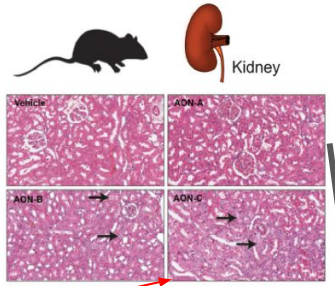
- The data platform Chan Zuckerberg CELL by GENE Discover (CZ CELLxGENE) provides data of 85 million cells as of April 2024 to be explored online.
- Much research and development now devotes to mapping data from different labs to reference datasets in order to annotate cells and samples in a (semi-)automated fashion



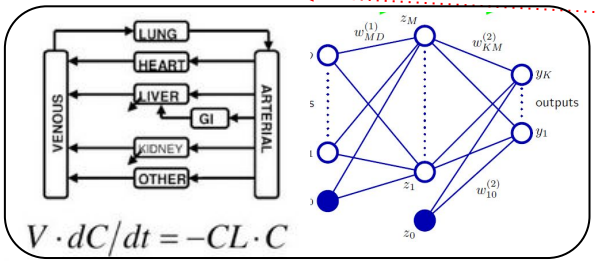
Computational methods empower efficacy and toxicity assessment





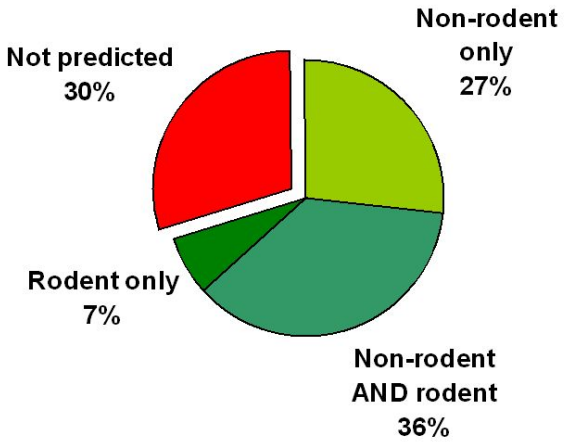


High-throughput technologies (omics, microscopy, etc.)



Mechanistic, causal, and statistical models

How predictive is animal safety testing for humans? It depends on modality and therapeutic classes.

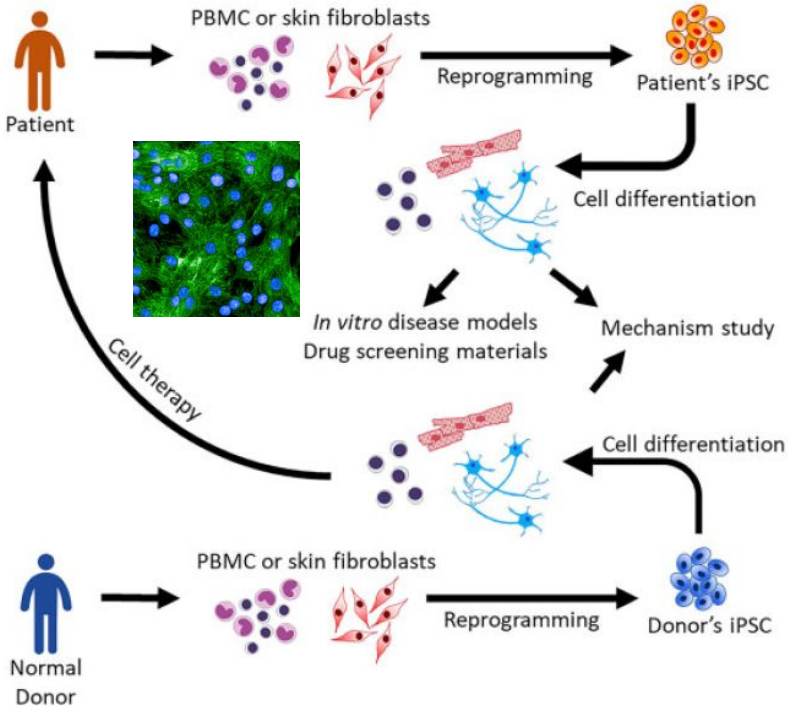


Regul Toxicol Pharmacol. 2000:32:56-67

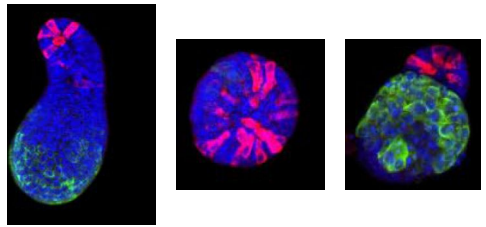
Target organ of ADRs	Small molecule drugs		Large molecule drugs	
	% of ADRs	% of correlation	% of ADRs	% of correlation
Gastrointestinal	21	80	14	19
Neurological	20	34	11	4
Hepatobiliary	11	73	8	21
Hematological	8	75	8	80
Cutaneous	5	56	9	22
Systemic	5	45	8	20
Cardiovascular	4	61	6	0
Ocular	5	64	5	83
Musculoskeletal	3	16	5	0
Metabolic	4	50	3	43
Faucal/oral	4	41	3	38
Urinary	3	61	3	14
Respiratory	1	45	5	32
Infection	0.4	100	6	68
Nasal	1	27	2	33
Application site reaction	1	100	3	81
Others	3	45	1	80

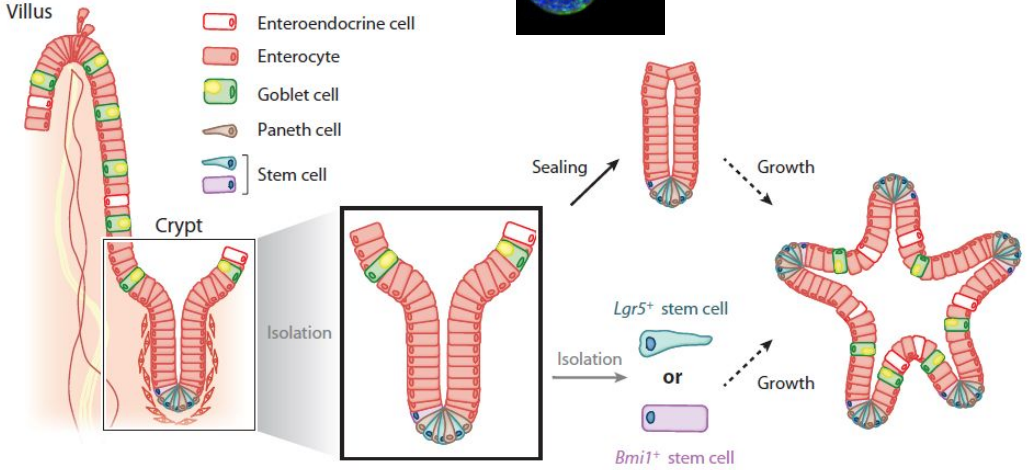
The Journal of Toxicological Sciences (J. Toxicol. Sci.)
Vol.38, No.4, 581-598, 2013

Stem cells and organoids empower efficacy and toxicity assessment



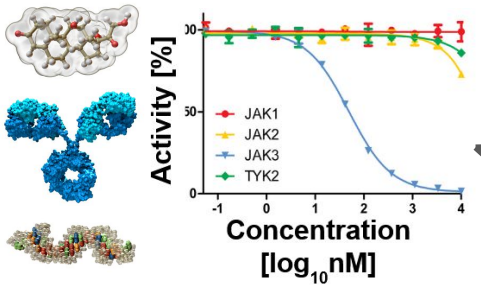
Induced pluripotent stem-cells

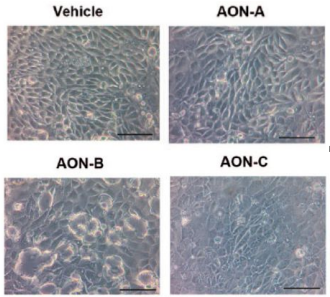


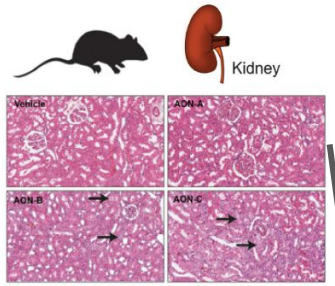


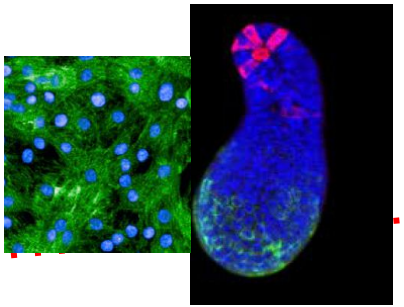
Small-intestinal organoids

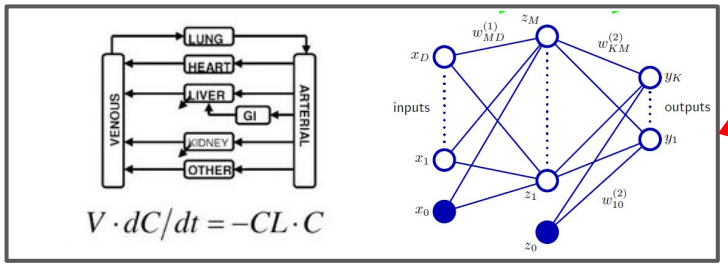
Computational methods and novel biological models empower efficacy and toxicity assessment











Conclusions

- We predict efficacy and safety profiles of drugs by studying the mechanism and mode of action (MoA).
- The study of MoA involves building mechanistic, statistical, and causal models to predict what drug does to the body (pharmacodynamics) and what body does to the drug (pharmacokinetics).
- Bulk and single-cell RNA sequencing, and proteomics based on mass spectrometry (MS, in backup) are essential tools for understanding MoA.

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1. Figures: Lumen Learning, Exploring Nature, National Geographics, Platelet cells (Graham Beards, CC-BY-SA 4.0), Lymphocytes (NicolasGrandjean, CC-BY-SA 3.0), Adipocytes (Public Domain), Hepatocytes (CC-BY-NC 2.0), Neurons and Glia (Public Domain), Blood (CC 3.0), Blood Cells (By A. Rad and M. Häggström. CC-BY-SA 3.0 license), A selective JAK3 inhibitor (London Lab/Weizmann institute)
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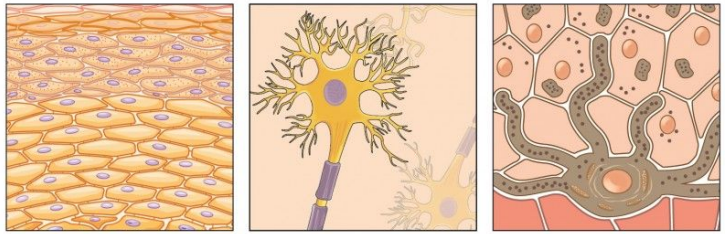
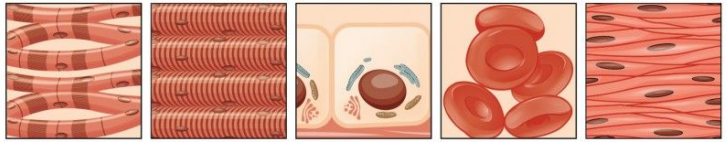
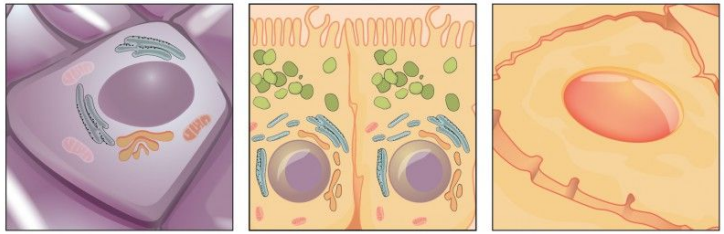
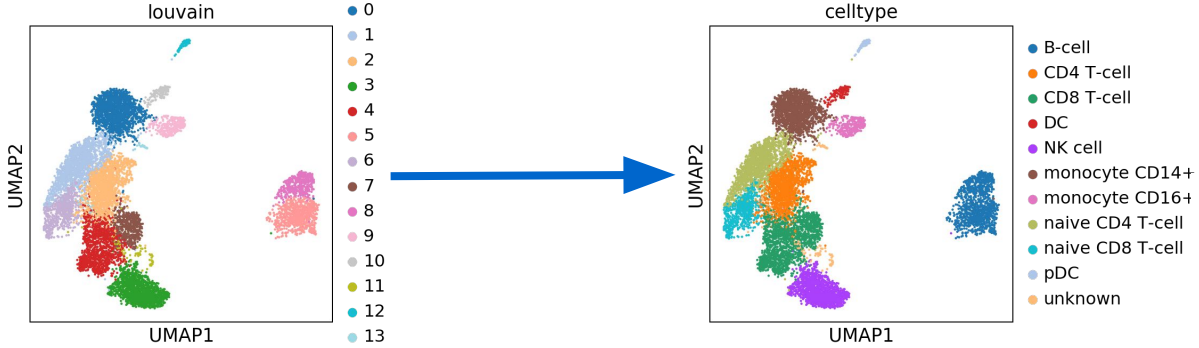
Supplementary Information

Embryonic origins of tissues

Germ Layer	Gives rise to:
Ectoderm	Epidermis, glands on skin, some cranial bones, pituitary and adrenal medulla, the nervous system, the mouth between cheek and gums, the anus  Skin cells Neurons Pigment cell
Mesoderm	Connective tissues proper, bone, cartilage, blood, endothelium of blood vessels, muscle, synovial membranes, serous membranes lining body cavities, kidneys, lining of gonads  Cardiac muscle Skeletal muscle Tubule cell of kidney Red blood cells Smooth muscle
Endoderm	Lining of airways and digestive system except the mouth and distal part of digestive system (rectum and anal canal); glands (digestive glands, endocrine glands, adrenal cortex)  Lung cell Thyroid cell Pancreatic cell

An intern project: Cell type annotation

From unsupervised clustering and cluster based annotation



Luis Wyss
RAAN intern 2019

	Gene 1	Gene 2	Gene 3	Gene 4	Gene 5	Label
Training Cell 1	10	50	0	12	4	Celltype A
Training Cell 2	8	45	78	3	23	Celltype B
Training Cell 3	14	55	78	65	55	Celltype B
Training Cell 4	78	12	13	9	58	Celltype A
Training Cell 5	45	23	65	98	11	Celltype C

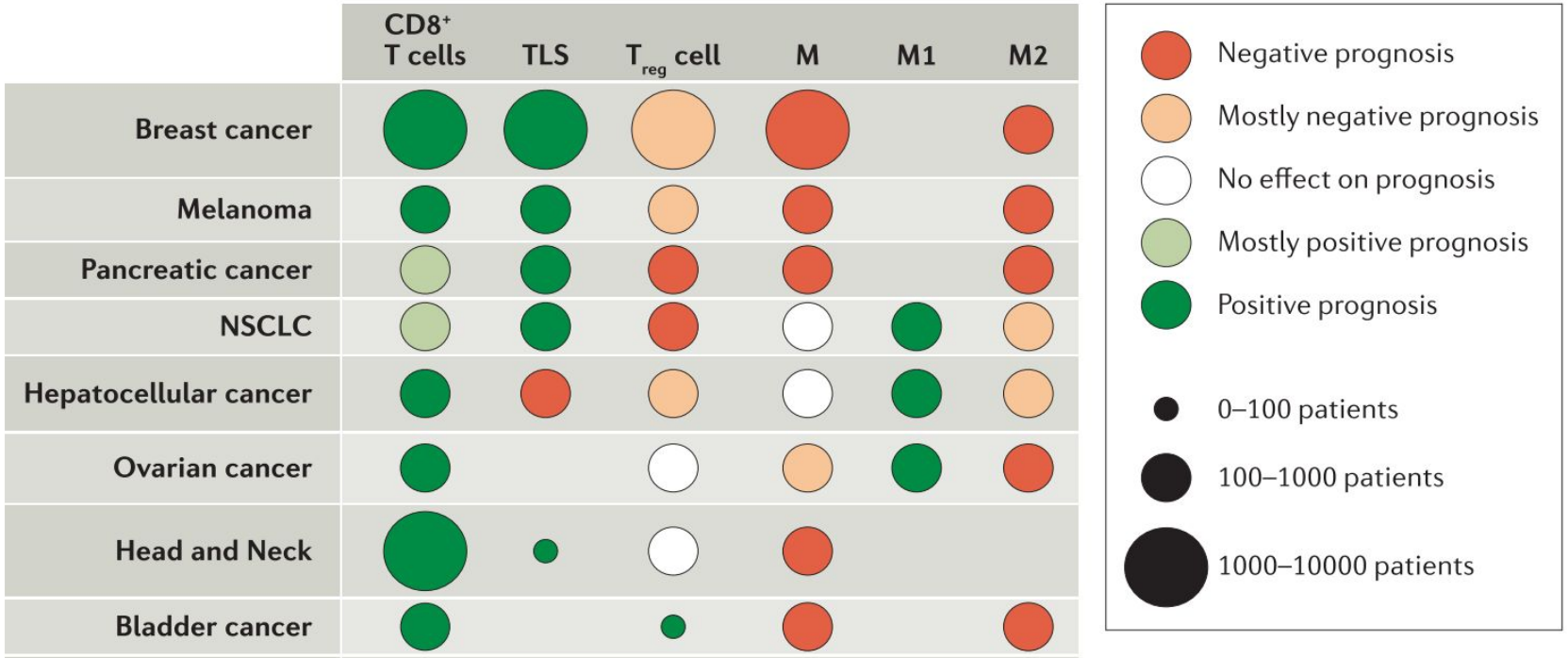
To supervised annotation at single-cell level:

	Gene 1	Gene 2	Gene 3	Gene 4	Gene 5
Cell 1	45	45	8	56	3
Cell 2	65	120	78	45	12
Cell 3	79	12	34	65	88
Cell 4	7	59	32	47	62

	Gene 1	Gene 2	Gene 3	Gene 4	Gene 5	Prediction
Cell 1	45	45	8	56	3	Celltype A
Cell 2	65	120	78	45	12	Celltype B
Cell 3	79	12	34	65	88	Celltype C
Cell 4	7	59	32	47	62	Celltype B

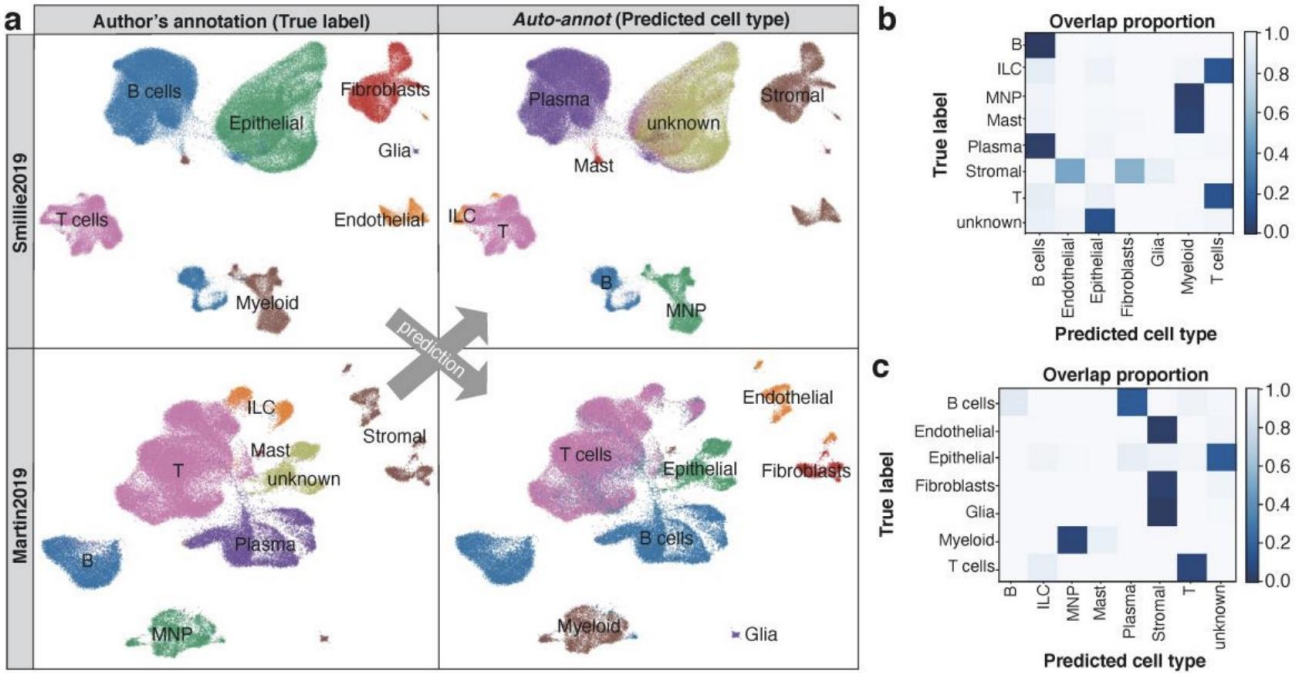
Advantages: (1) automation, (2) annotation independent from clustering, and (3) we can estimate the confidence of prediction

Abundance of immune cells in tumor microenvironments affect outcome



TLS: tertiary lymphoid structures; T_{reg}: regulatory T cells; M: macrophages; M1/M2: subtypes of macrophages

An example of Inflammatory Bowel Disease (IBD)



We observed Inconsistent cell type nomenclature across studies.
Machine learning allows us compare and integrate multiple studies.

We are living ecosystems

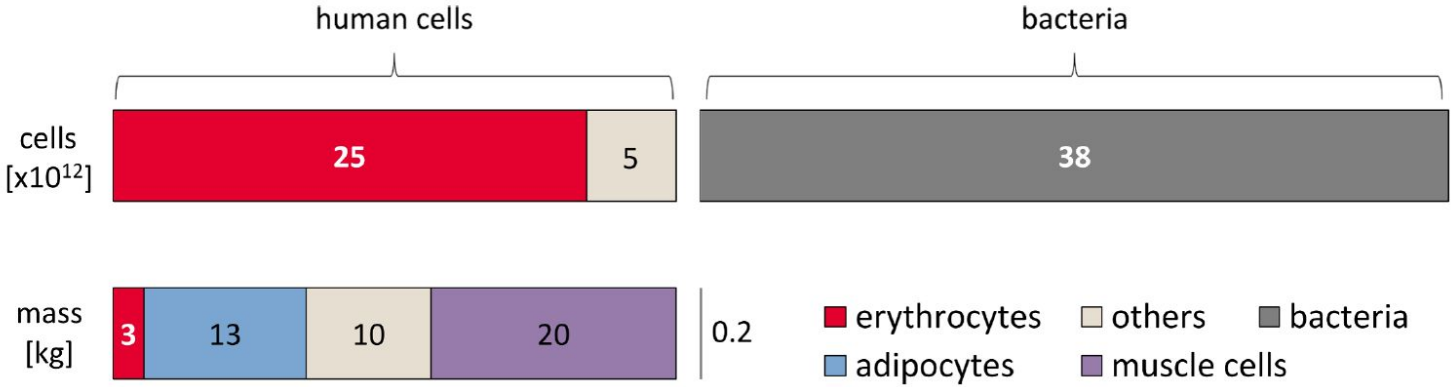
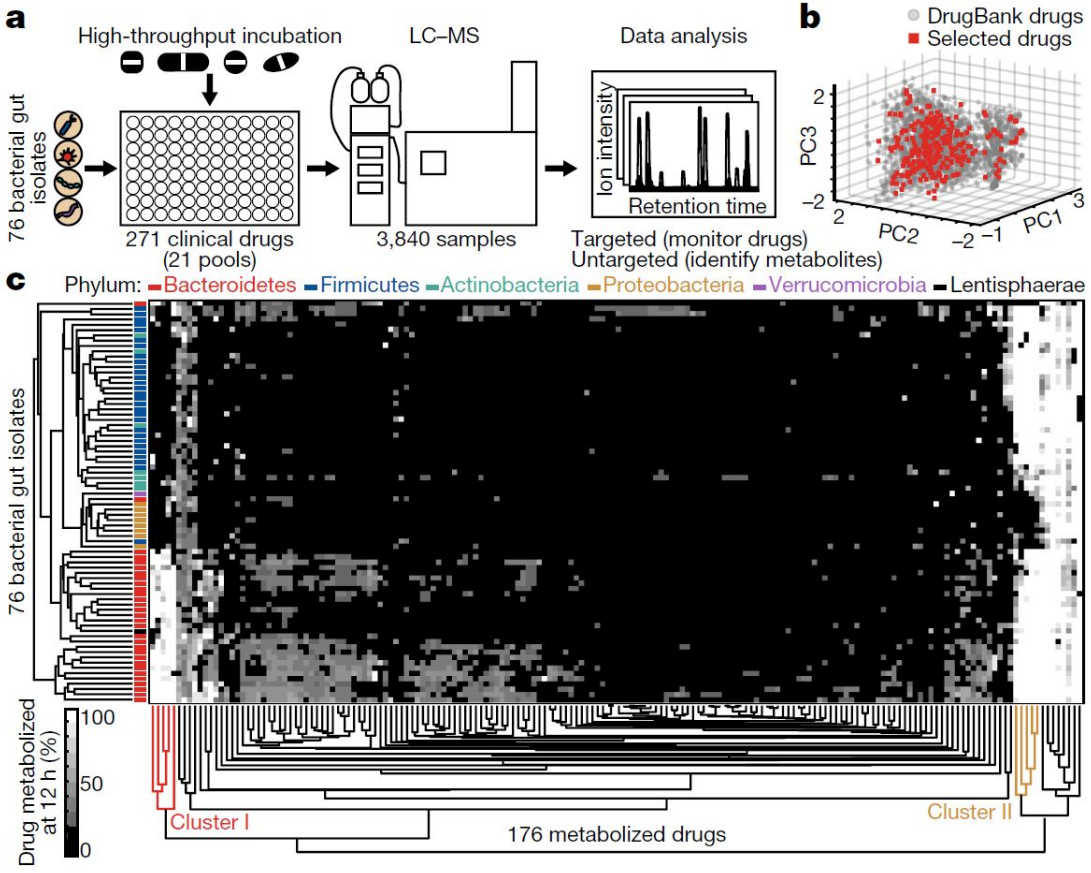
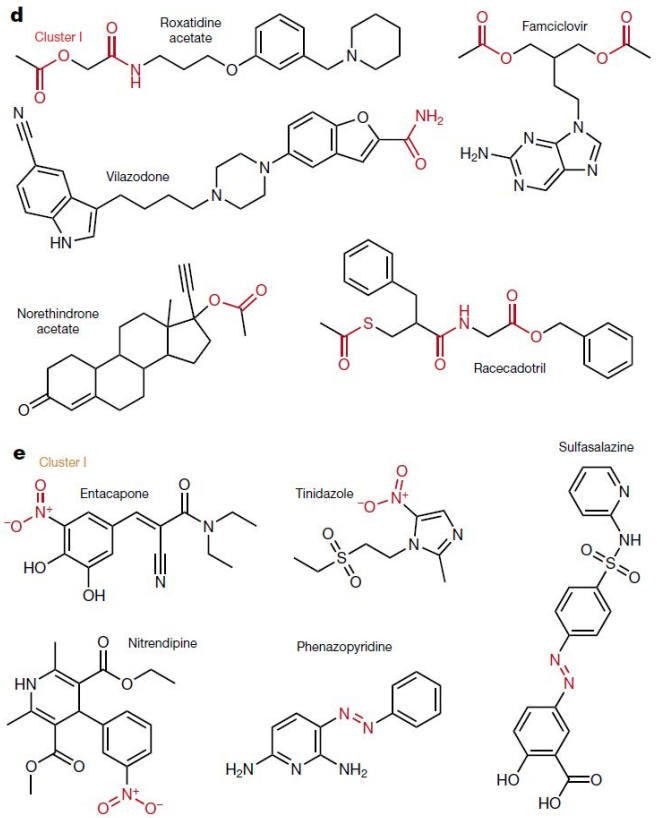


Table 3. B/H ratio for different population. See Table B in S1 Appendix for full references.

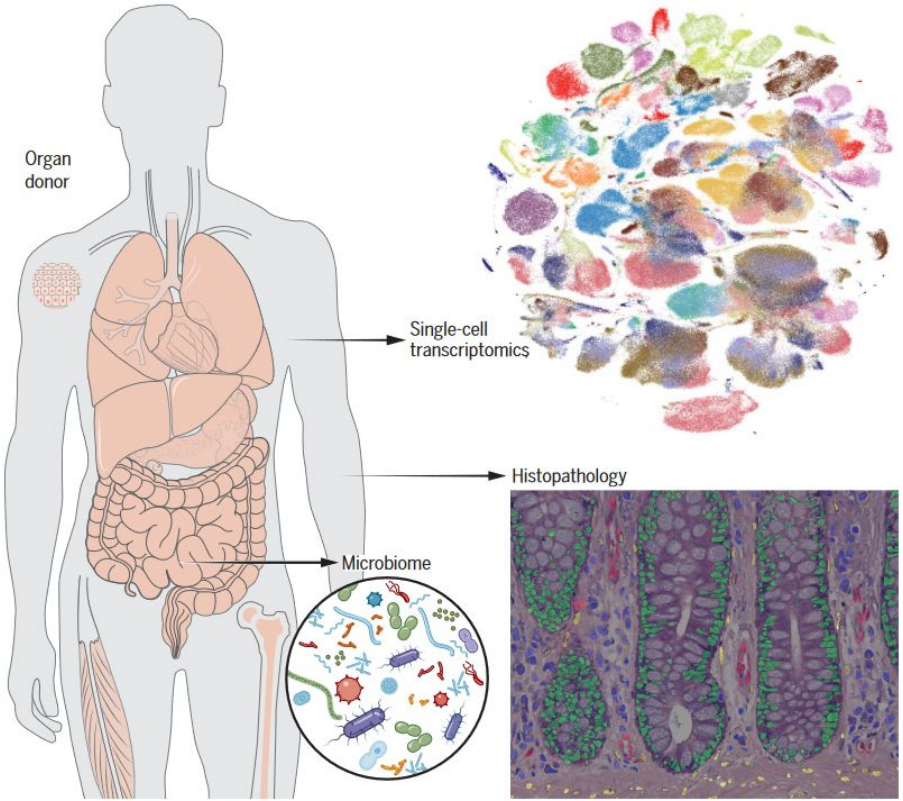
population segment	body weight [kg]	age [y]	blood volume [L]	RBC count [10^{12}/L]	colon content [g]	bac. conc. [10^{11}/ g wet] (1)	total human cells [10^{12}] (2)	total bacteria [10^{12}]	B:H
ref. man	70	20–30	4.9	5.0	420	0.92	30	38	**1.3**
ref. woman	63		3.9	4.5	480	0.92	21	44	**2.2**
young infant	4.4	4 weeks	0.4	3.8	48	0.92	1.9	4.4	**2.3**
infant	9.6	1	0.8	4.5	80	0.92	4	7	**1.7**
elder	70	66	3.8 (3)	4.8	420	0.92	22	38	**1.8**
obese	140		6.7	5.0 (4)	610 (5)	0.92	40	56	**1.4**

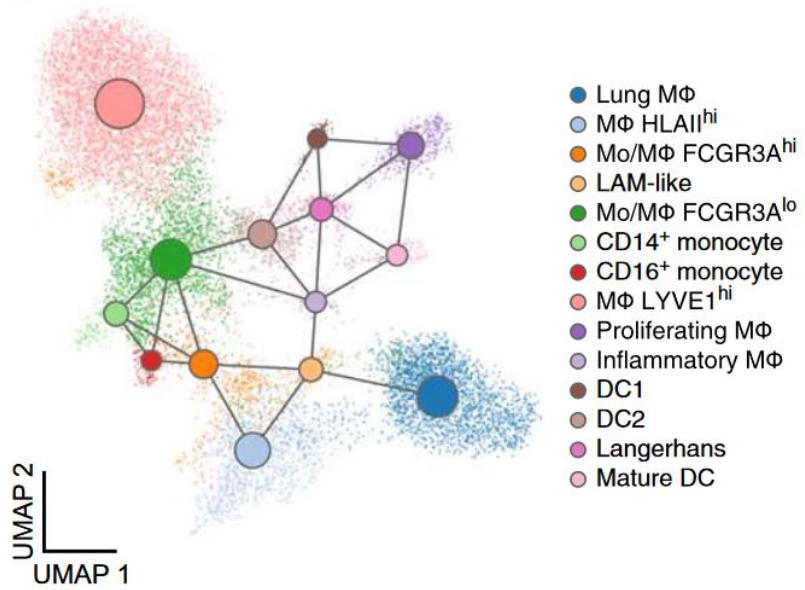
Gut microbiome can metabolize drugs differently





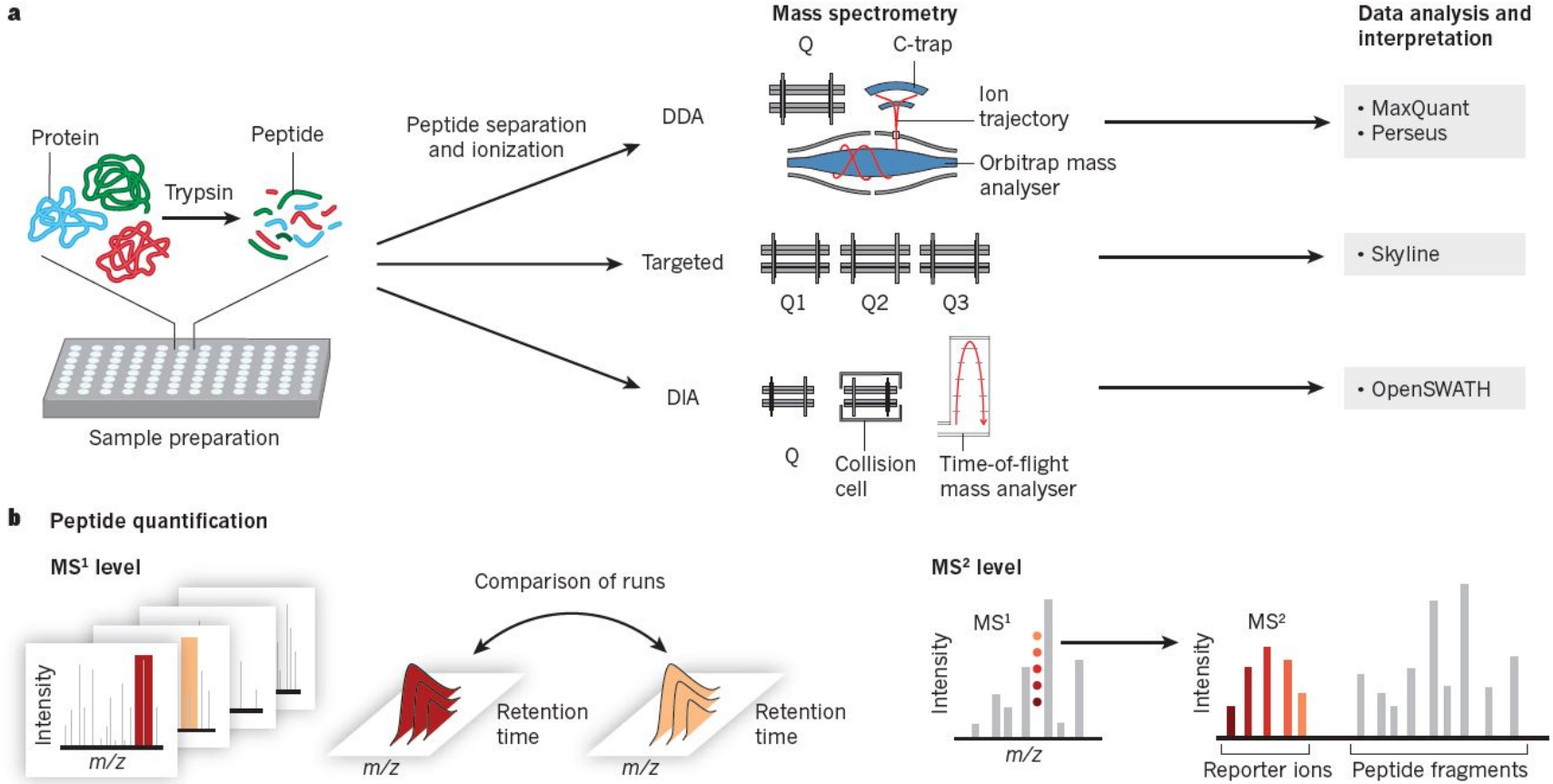
The *Tabula Sapiens* and other community projects offer reference expression data in healthy donors



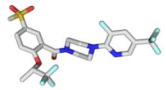


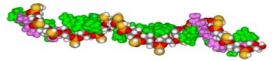
Left: the *Tabula Sapiens*. Right: Myeloid (Mϕ=macrophages, Mo/monocytes, LAM=lipid-associated macrophages, DC=dendritic cells) gene expression

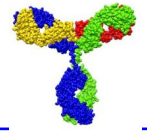
Mass-spectrometry based proteomics



Comparing modalities with regard to safety assessment



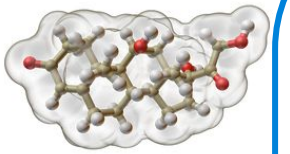


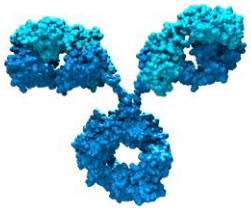


	Small molecules	Single Stranded Oligos	Biologics
Molecular weight	<1000 D	5000-7000 D	> 30000 D
Manufacture	Chemical synthesis	Chemical synthesis	Biologically-derived
Structure	Single entity, high purity	Single entity with 10-15% product-related impurities	Complex, heterogeneous
Chemical-driven toxicity	Yes	Yes	No
Metabolism	Species-specific	Species-independent catabolism by proteolytic degradation	Species-independent catabolism by proteolytic degradation
PK	Generally short $t_{1/2}$	Long (tissue) $t_{1/2}$	Long $t_{1/2}$
Some general aspects	High throughput screening/early safety testing of up to 500 small molecules	Biodistribution with consistent patterns	Fewer, yet complex due to biology/immunology

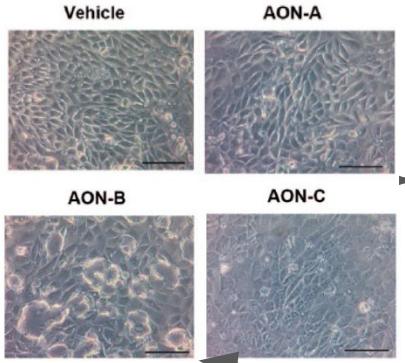
Adapted from Schubert et al, Nucl Acid Therap 2012, with input from Yann Tessier and Susanne Mohr

Proteomics plays an important role in *in vitro*/*in vivo* translation

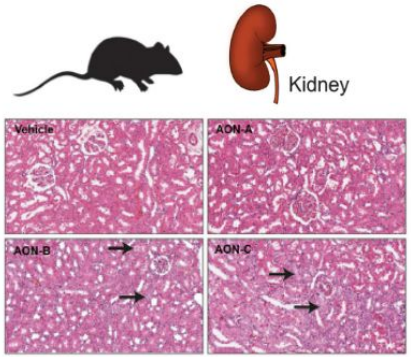




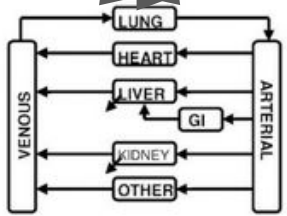
in vitro



in vivo



Clinical trial

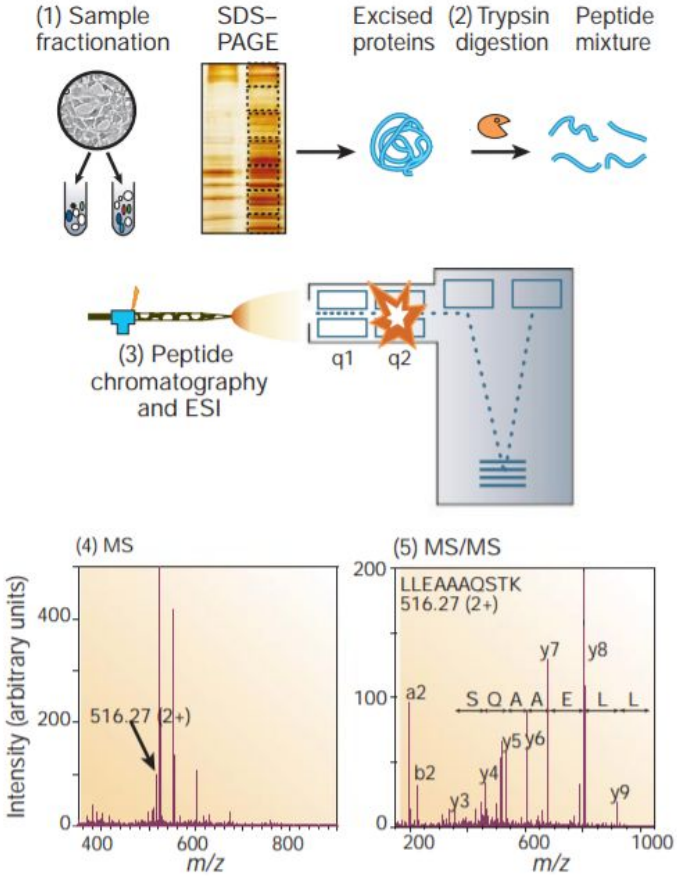


in silico

$$V \cdot dC/dt = -CL \cdot C$$

Mass-spectrometry based proteomics

- **SDS-PAGE**: Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
- **ESI**: Electrospray ionization
- **q1/q2**: selection/collision/separation cells
- **MS**: Mass spectrometry
- **MS/MS**: tandem mass spectrometry



Proteomics approaches for drug discovery

Affinity purification

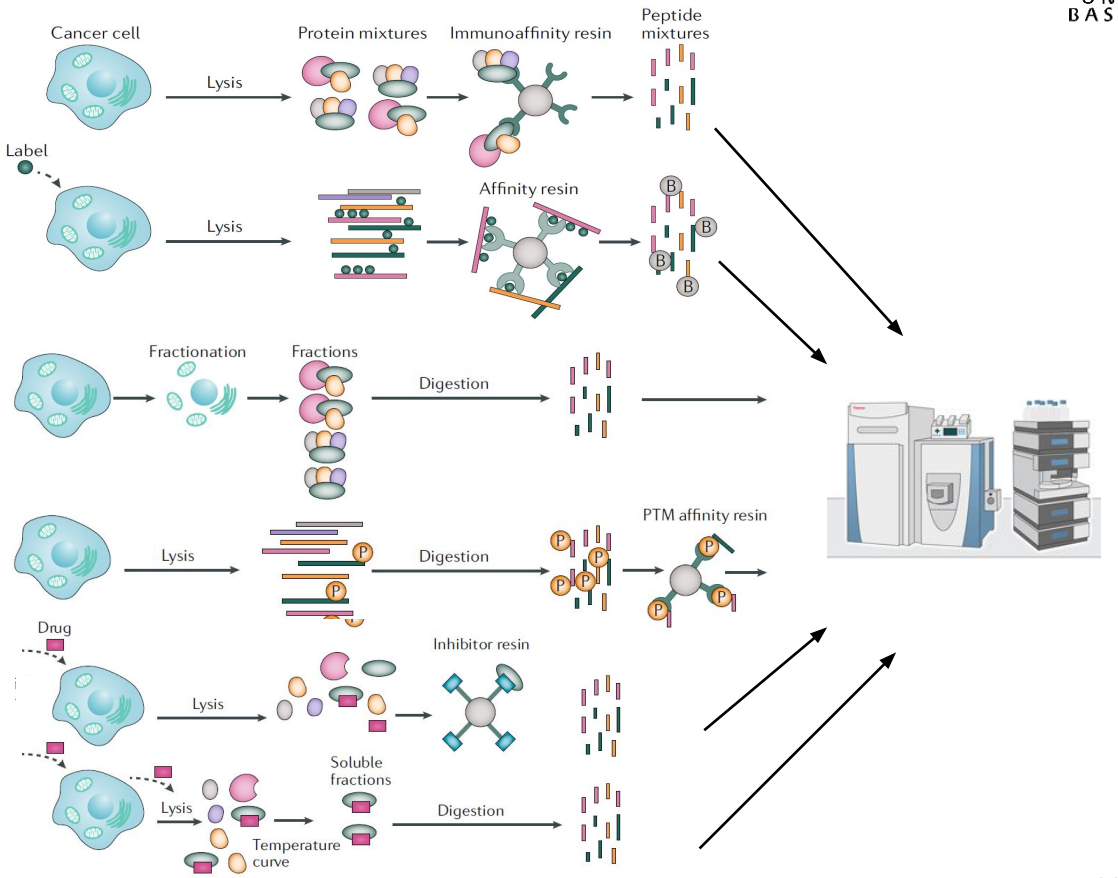
Proximity labelling

Organelle proteome profiling

Post-translational modification (PTM) profiling

Chemoaffinity enrichment

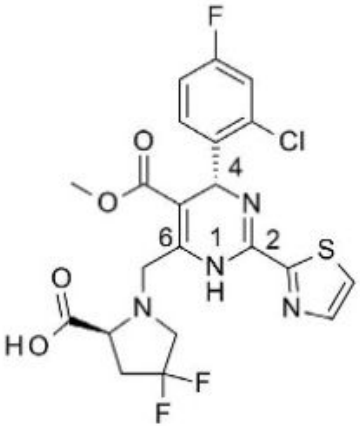
Thermal proteome profiling



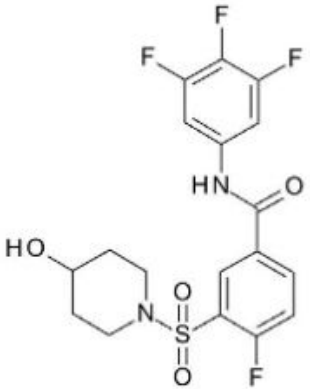
Case 1: Differentiate two compounds that inhibit Hepatitis B Virus with similar mode of action

a

HAP_R01

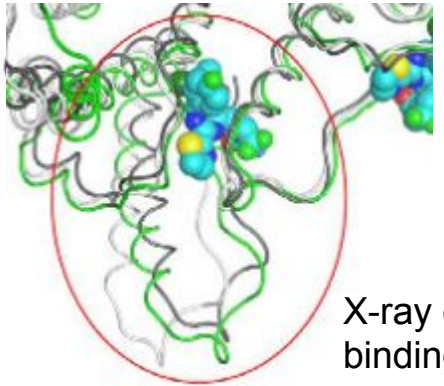


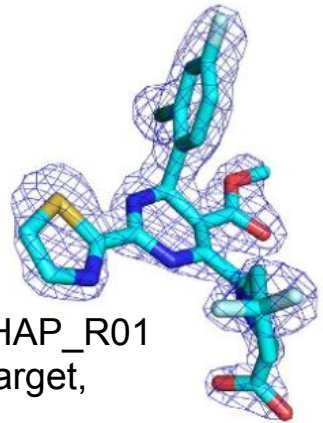
SBA_R01



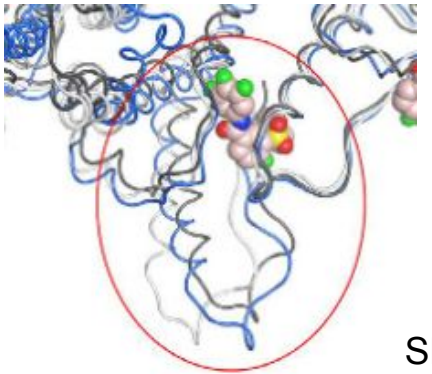
b

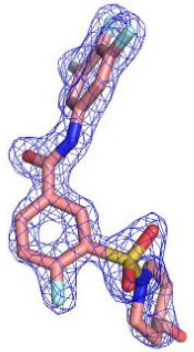
Compound	IC_{50} (μM)	HepG2.2.15 EC_{50} (μM)	CC_{50} (μM)
HAP_R01	0.39±0.13	0.0064±0.0006	34.8±1.8
SBA_R01	1.90±0.22	0.26±0.02	8.05±0.92





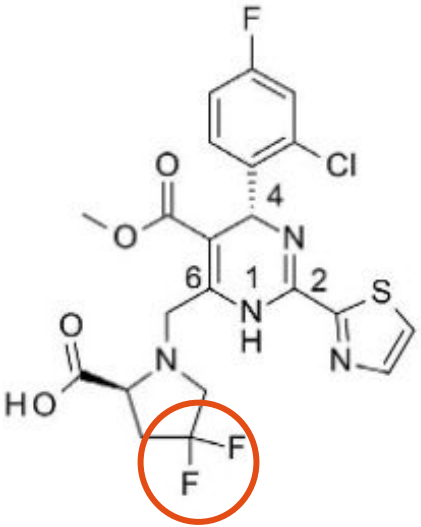
X-ray data of HAP_R01 binding to its target, HBV capsid



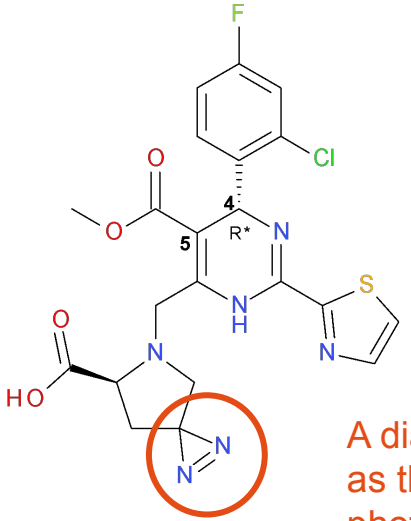


SBA_R01

Chemical probes: drug-like molecules to probe its mode of action

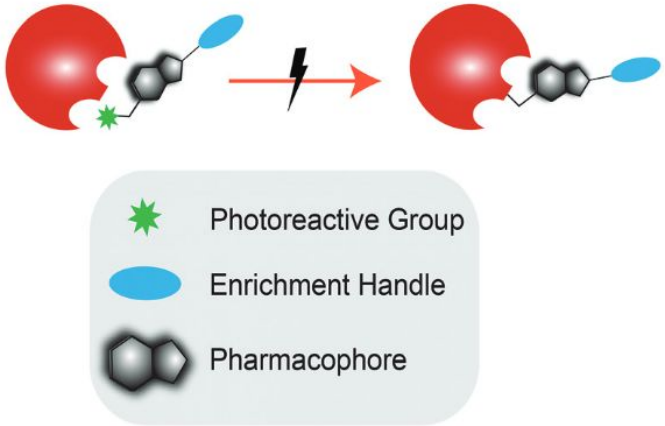


Compound	IC_{50} (μM)
HAP_R01	0.39±0.13

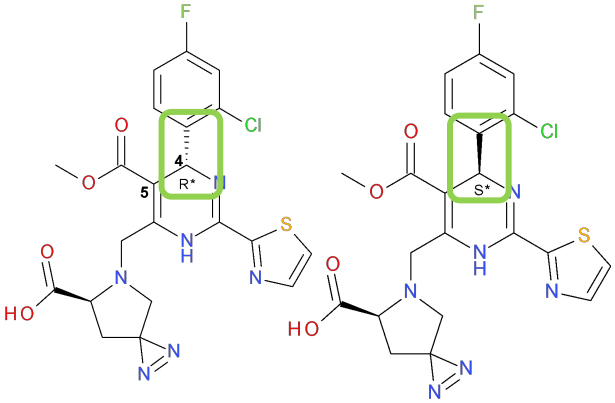


RO-A
EC_{50}: **0.040 μM**
IC_{50}: **0.47 μM**

A diazirine group as the photoreactive group



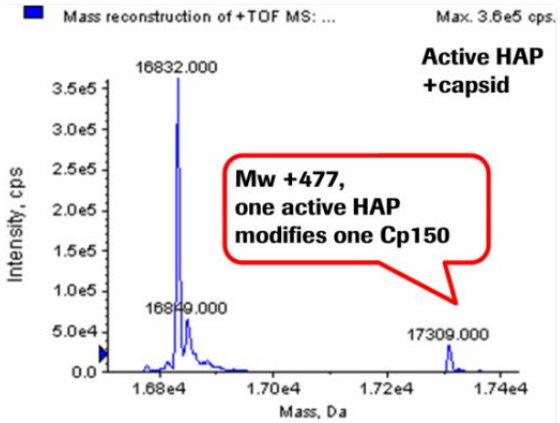
Case 1 solved: Proteomics confirmed target binding and mapped the small molecule binding pocket

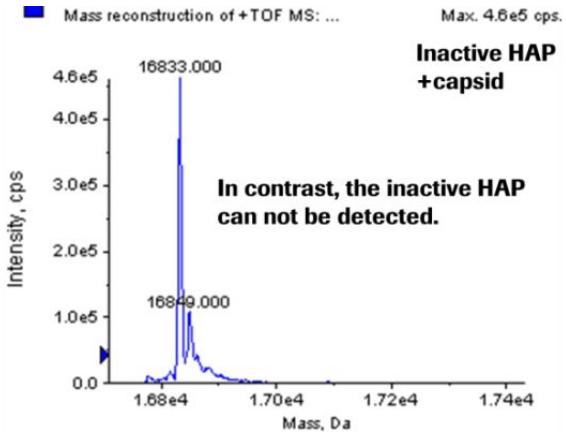


RO-A
EC_{50}: **0.040 µM**
IC_{50}: **0.47 µM**

RO-B
EC_{50}: **>1 µM**
IC_{50}: **>100 µM**

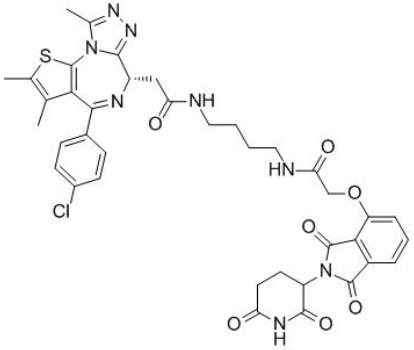
+Cp150, UV, MS



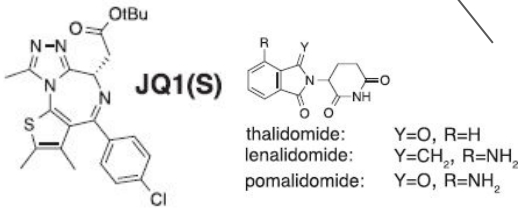


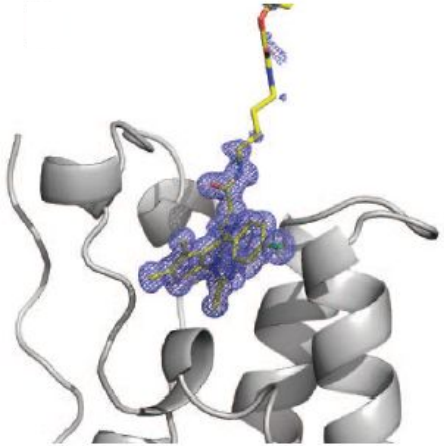
Proteolytic digestion/LC-MS/MS identified labelling site Y118 (Y=Tyrosine) of HBV capsid protein. More photoaffinity probes identified labelling sites at R127 (R=Arginine) and Y38.

Case 2: Confirmation of selective degradation of protein target *in vivo*

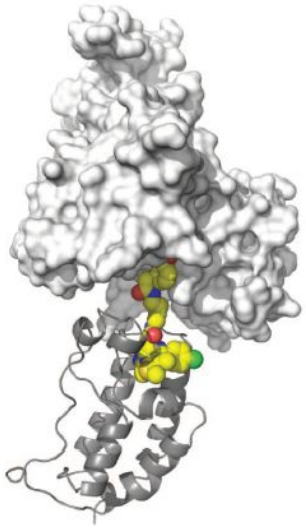


dBET1

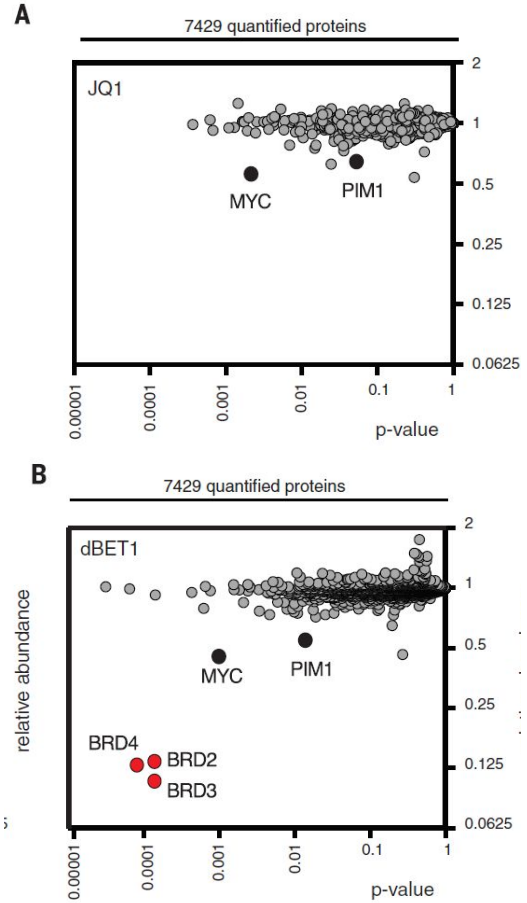




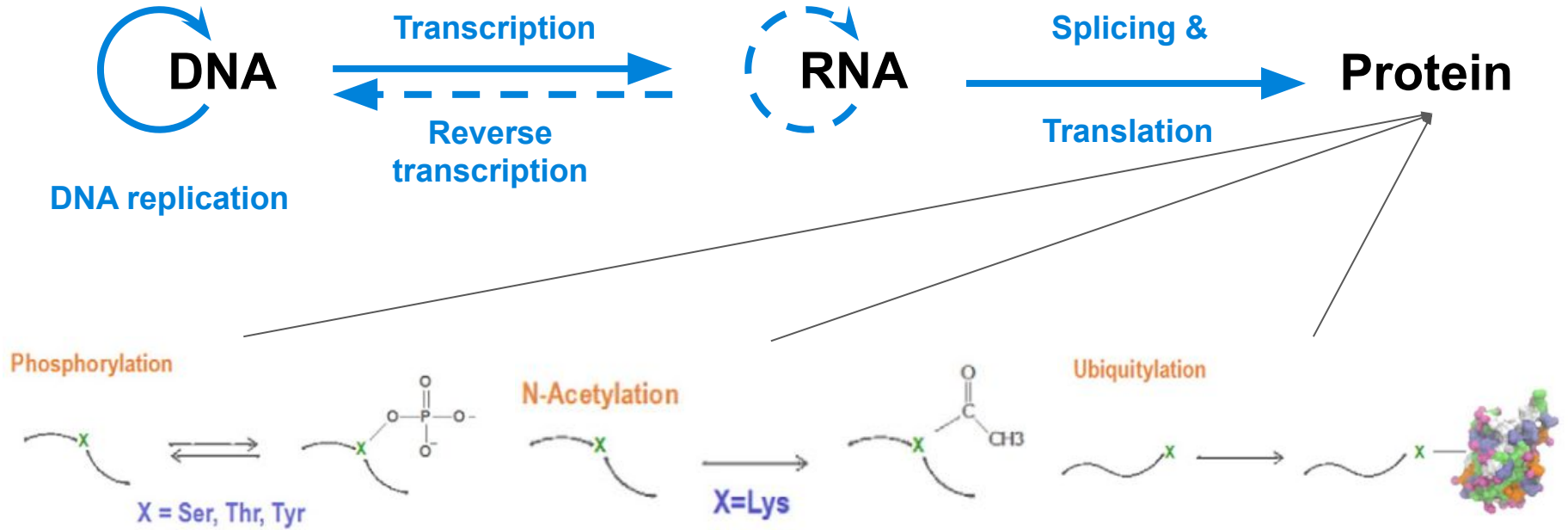
Crystal structure of dBET1 binding to its target BRD4



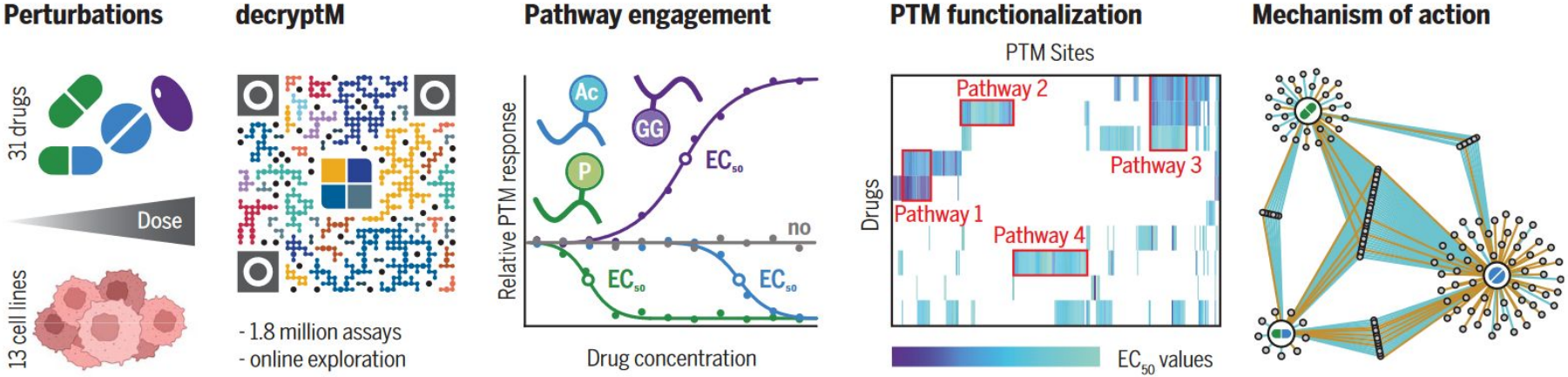
Docking of dBET1-BRD4 to DDB1-CRBN structure



Protein post-translational modifications (PTMs) offer an additional layer of regulation

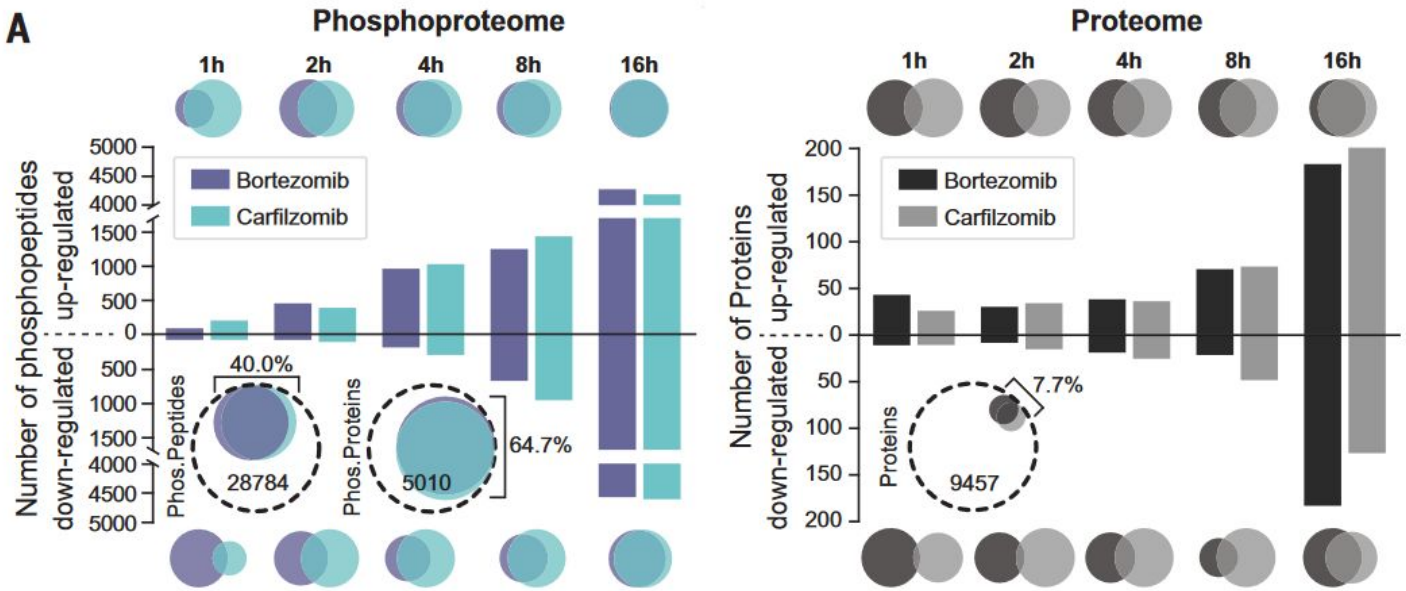


Case 3: Millions of PTM profiles induced by drugs in cancer cell lines



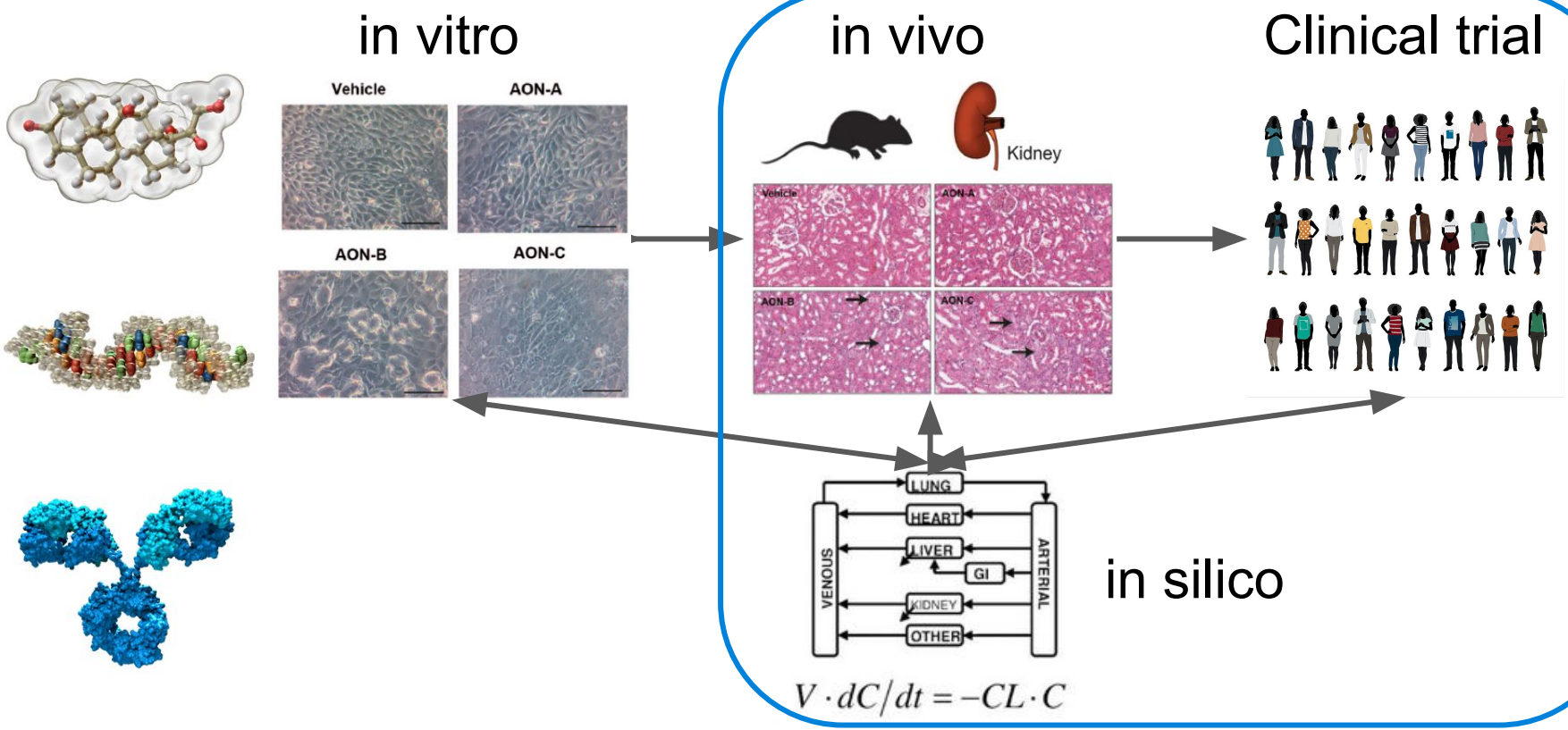
decryptM (Nature 2023): Following the dose-dependent treatment of cancer cells with drugs, quantitative mass spectrometry records dose-response of thousands of posttranslationally modified peptides. EC50: half-maximal effective concentration; Ac, acetylation; GG, ubiquitinylation; P, phosphorylation.

PTM and proteomics characterize MoA of drugs

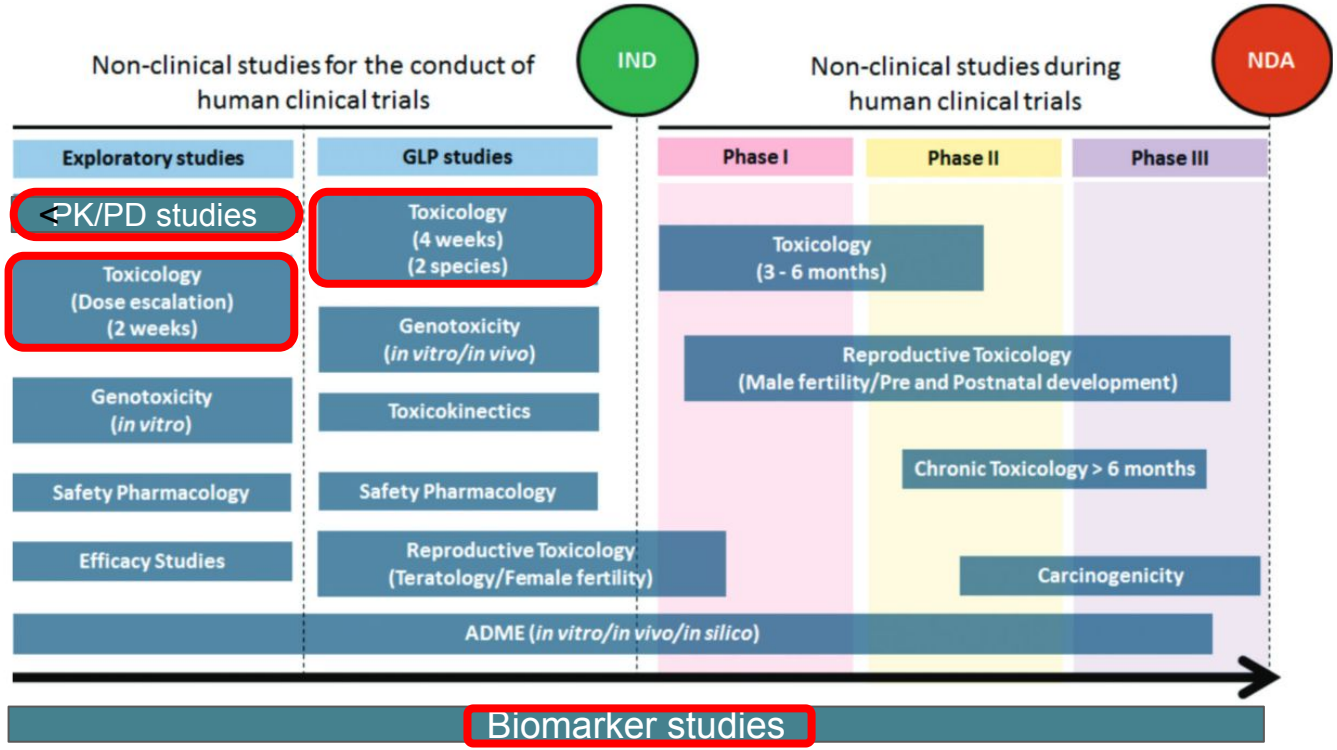


Bortezomib (BTZ) and carfilzomib (CFZ) both treat multiple myeloma by inhibiting the proteasome by reversible covalent (BTZ) or irreversible (CFZ) binding to the protease PSMB5. Time-series data show both the dynamics and the converging signaling.

Dose prediction based on pharmacology and toxicology before entry into human

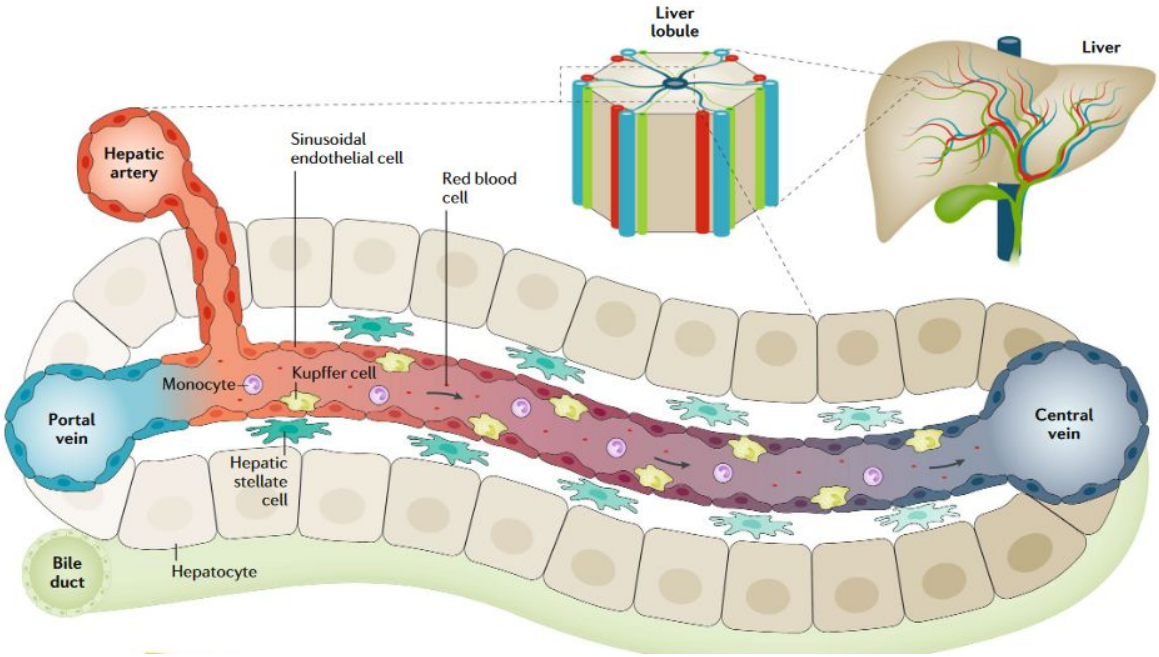


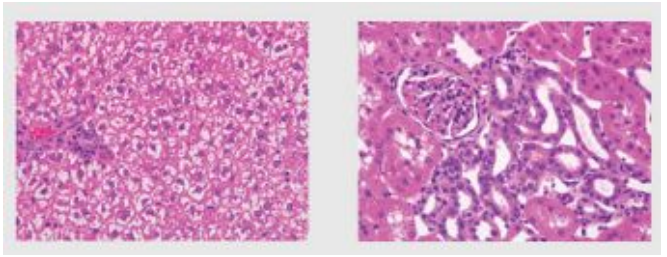
Current practices of non-clinical studies in drug development



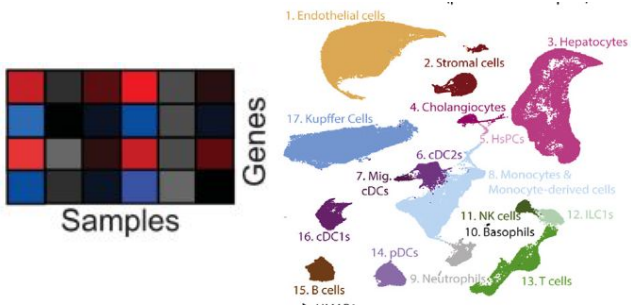
- IND: Investigational New Drug application
- NDA: New Drug Application
- GLP: Good Lab Practice
- Red boxes: Focus areas of this and coming lectures

Current practices of profiling and understanding toxicology: an example with liver





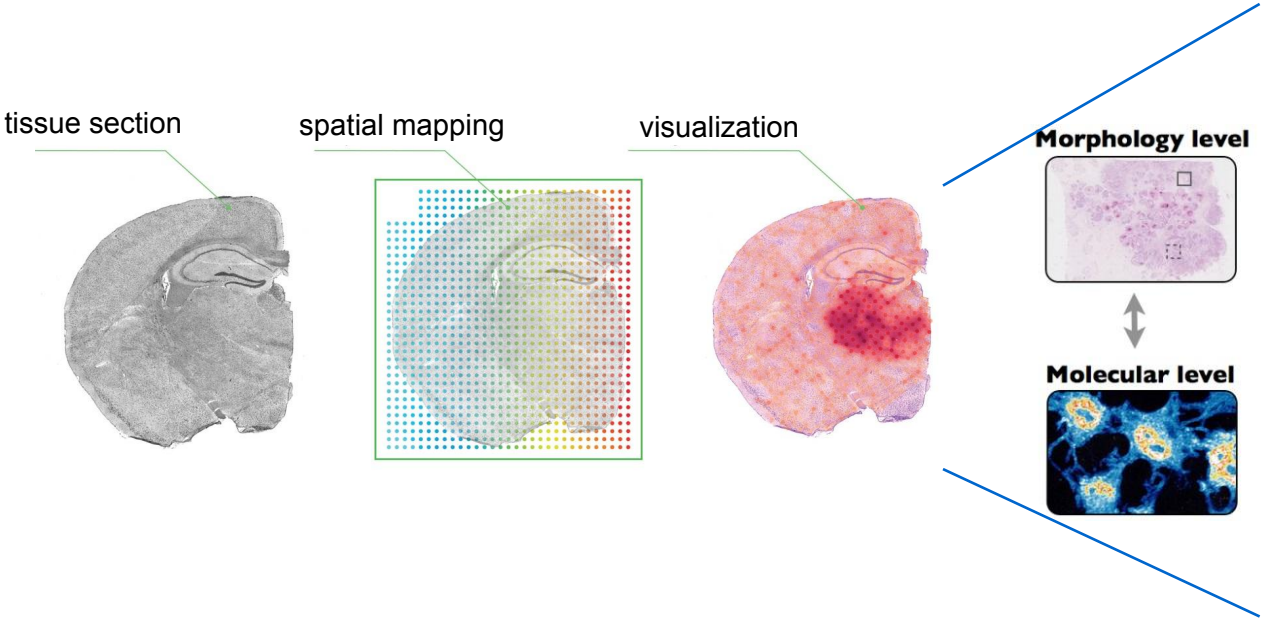
Histopathology

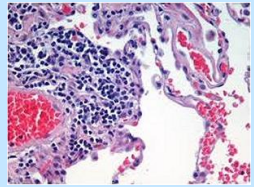


Omics

[Liver structure and anatomy (YouTube Video)]

Spatially resolved omics complement histopathology





Spatial Omics

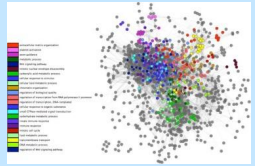
Histopathology

- Morphology

Molecular Biomarkers

- Genetics, Transcriptomics

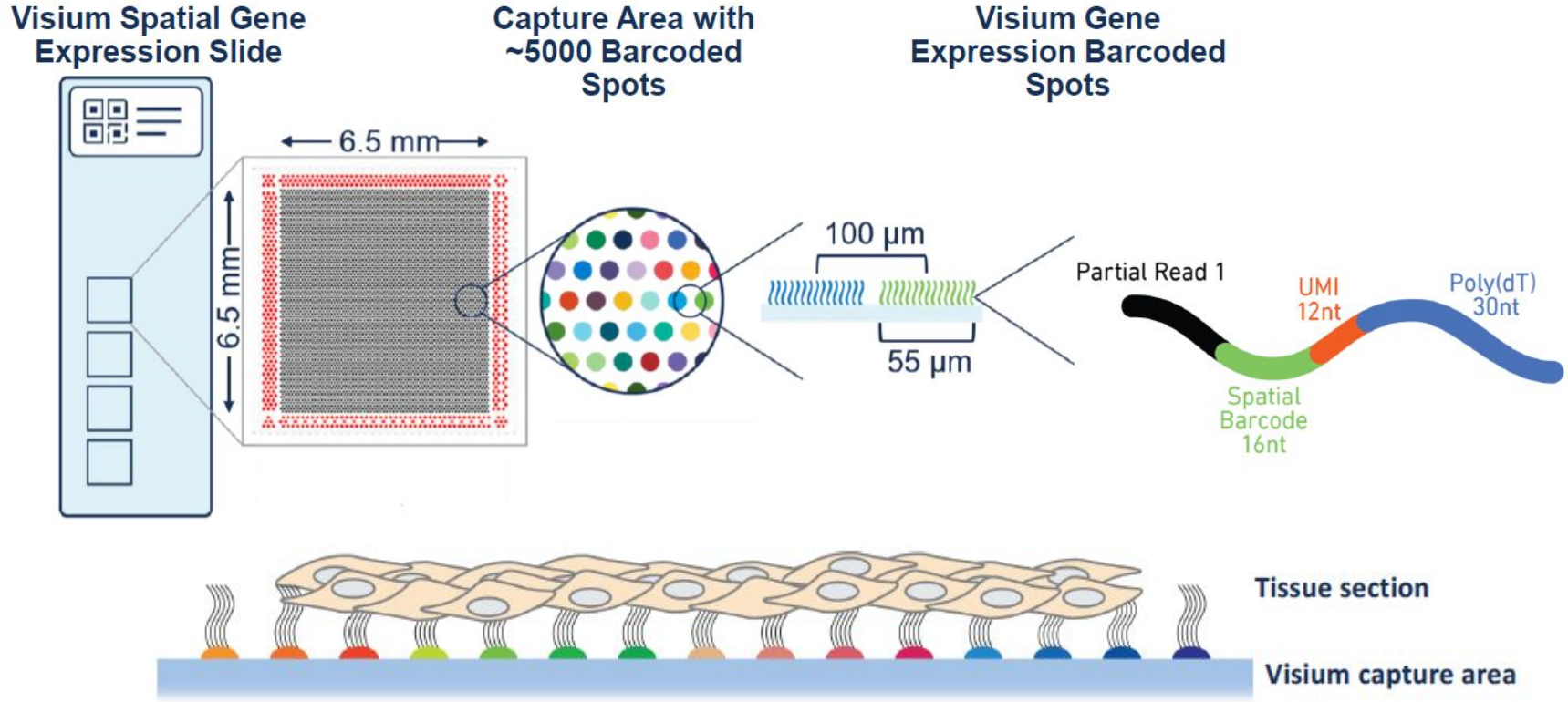
- Proteomics



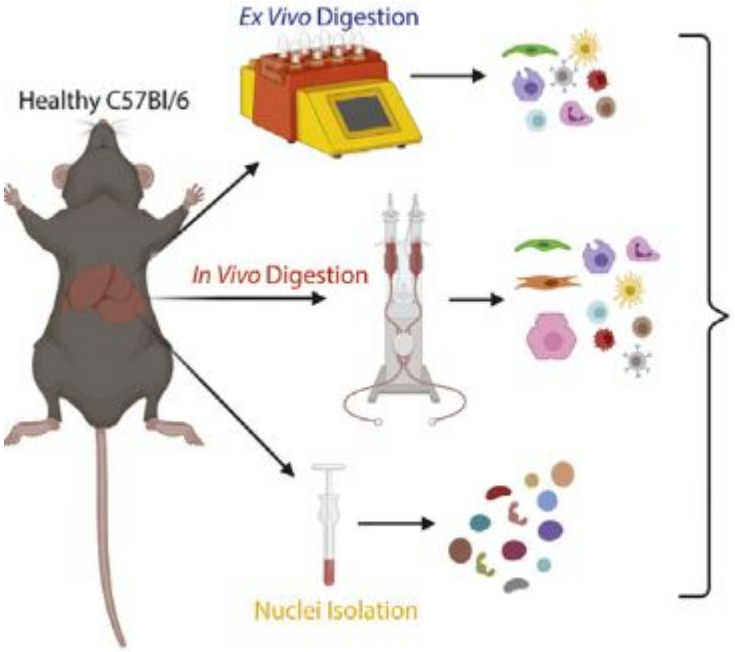
Data insights

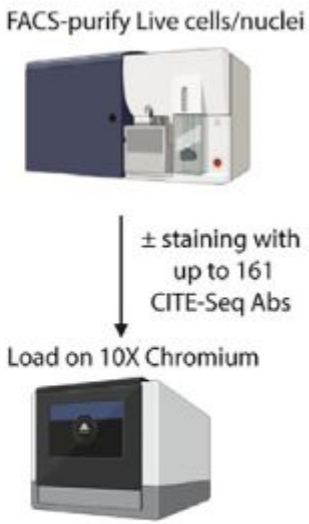
- Data analysis & integration

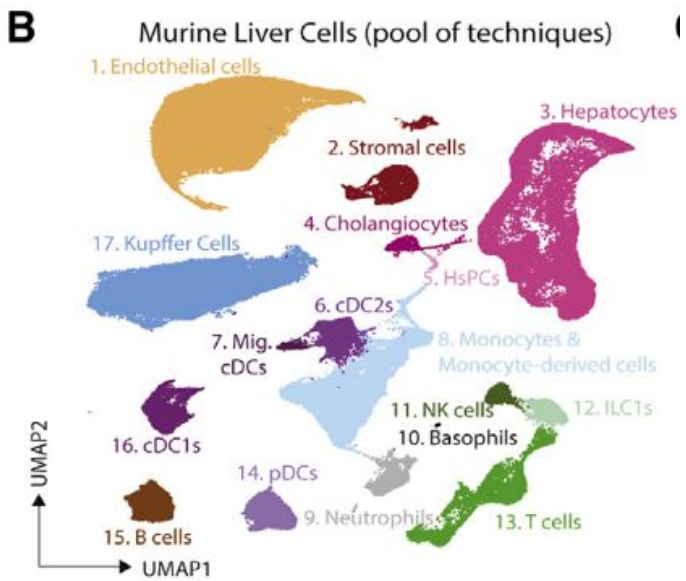
An example: 10x VISIUM Technology



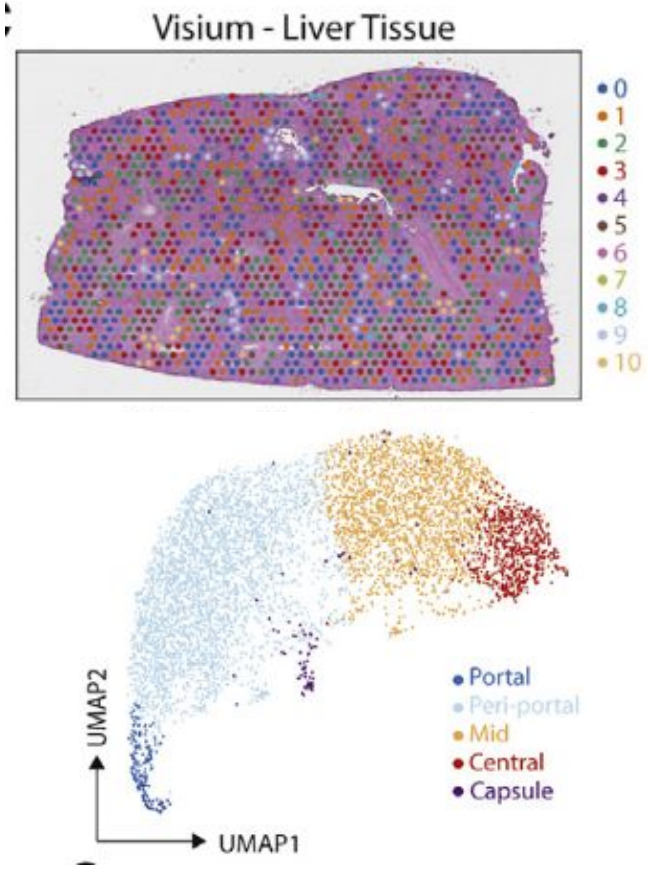
Spatial and single-cell expression of liver cells

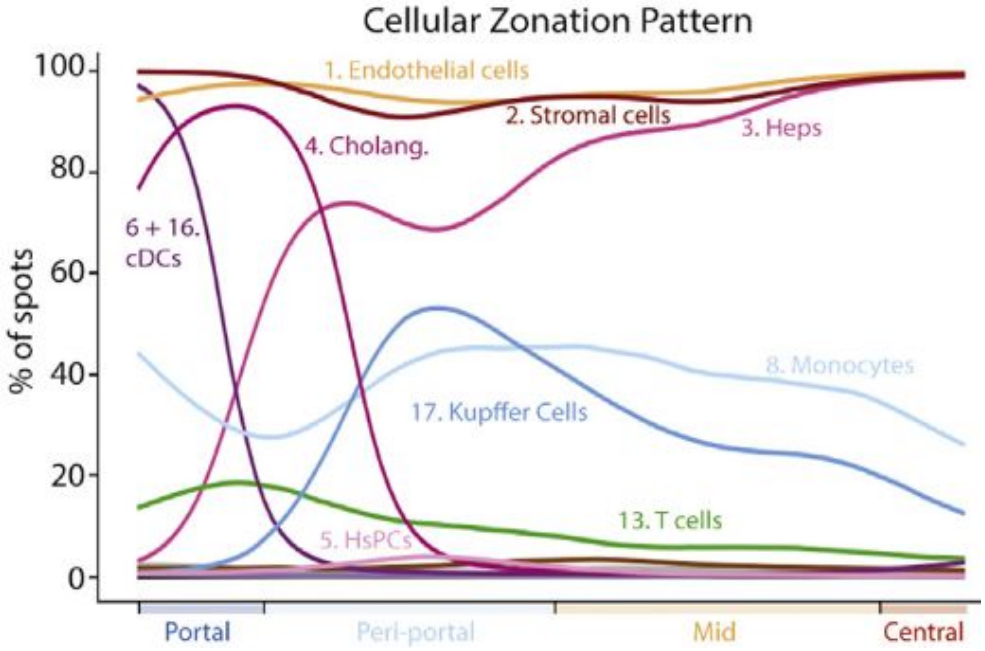




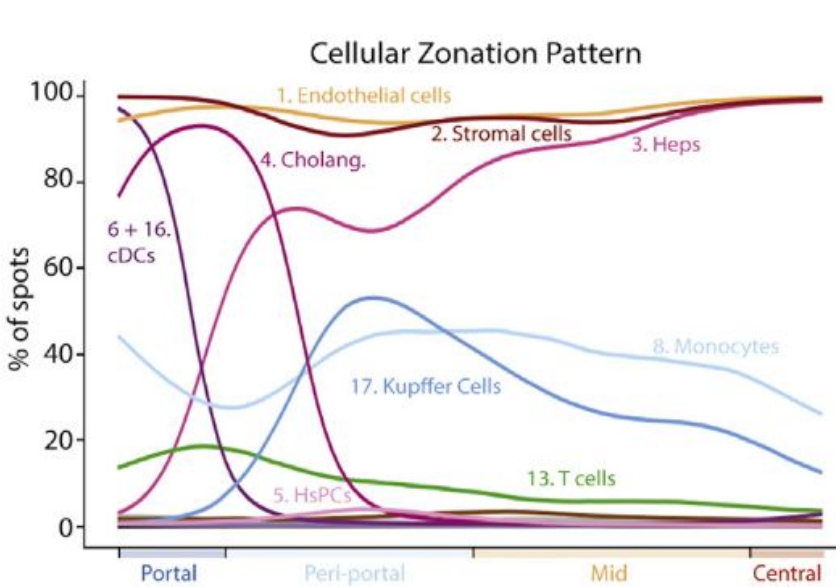


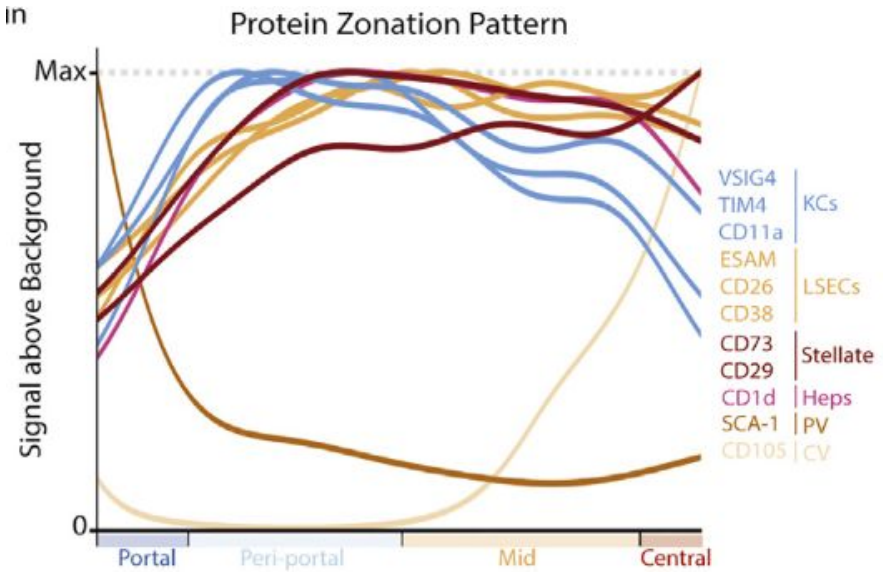
Spatial and single-cell expression of liver cells





Spatial mRNA and protein expression data empowers digital pathology and biological understanding





Proteomics enables the elucidation of protein relations in the protein communities